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(54) Title: THERMITASE VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

(57) Abstract

The present invention relates to Thermitase variants having a modified amino acid sequence of wild-type Thermitase amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Thermitase (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Thermitase variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Thermitase. The present invention also relates to DNA sequences encoding such Thermitase variants. The present invention also relates to compositions comprising such Thermitase variants for cleaning a variety of surfaces.

THERMITASE VARIANTS HAVING DECREASED ADSORPTION
AND INCREASED HYDROLYSIS

TECHNICAL FIELD

The present invention relates to novel enzyme variants useful in a variety of cleaning compositions, and DNA sequences encoding such enzyme variants.

BACKGROUND

Enzymes make up the largest class of naturally occurring proteins. Each class of enzyme generally catalyzes (accelerates a reaction without being consumed) a different kind of chemical reaction. One class of enzymes known as proteases, are known for their ability to hydrolyze (break down a compound into two or more simpler compounds with the uptake of the H and OH parts of a water molecule on either side of the chemical bond cleaved) other proteins. This ability to hydrolyze proteins has been taken advantage of by incorporating naturally occurring and protein engineered proteases as an additive to laundry detergent preparations. Many stains on clothes are proteinaceous and wide-specificity proteases can substantially improve removal of such stains.

Unfortunately, the efficacy level of these proteins in their natural, bacterial environment, frequently does not translate into the relatively unnatural wash environment. Specifically, protease characteristics such as thermal stability, pH stability, oxidative stability and substrate specificity are not necessarily optimized for utilization outside the natural environment of the enzyme.

The amino acid sequence of the protease determines the characteristics of the protease. A change of the amino acid sequence of the protease may alter the properties of the enzyme to varying degrees, or may even inactivate the enzyme, depending upon the location, nature and/or magnitude of the change in the amino acid sequence. Several approaches have been taken to alter the wild-type amino acid sequence of proteases in an attempt to improve their properties, with the goal of increasing the efficacy of the protease in the wash environment. These approaches include altering the amino acid sequence to enhance thermal stability and to

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improve oxidation stability under quite diverse conditions.

Despite the variety of approaches described in the art, there is a continuing need for new effective variants of proteases useful for cleaning a variety of surfaces.

Objects of the Present Invention

It is an object of the present invention to provide Thermitase enzyme variants having improved hydrolysis versus the wild-type of the enzyme.

It is also an object of the present invention to provide cleaning compositions comprising these subtilisin enzyme variants.

SUMMARY

The present invention relates to Thermitase variants having a modified amino acid sequence of wild-type Thermitase amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region and a fifth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Thermitase (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Thermitase variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Thermitase. The present invention also relates to DNA sequences encoding such Thermitase variants. The present invention also relates to compositions comprising such Thermitase variants for cleaning a variety of surfaces.

DESCRIPTION

I. Thermitase Variants

This invention pertains to subtilisin enzymes, in particular Thermitase, that have been modified by mutating the various nucleotide sequences that code for the enzyme, thereby modifying the amino acid sequence of the enzyme. The modified subtilisin enzymes (hereinafter, "Thermitase variants") of the present invention have decreased adsorption to and increased hydrolysis of an insoluble substrate as compared to the wild-type subtilisin. The present invention also pertains to DNA sequences encoding for such Thermitase variants.

The subtilisin enzymes of this invention belong to a class of enzymes known as proteases. A protease is a catalyst for the cleavage of peptide bonds. One type of protease is a serine protease. A serine protease is distinguished by the fact that there is an essential serine residue at the

active site.

The observation that an enzyme's rate of hydrolysis of soluble substrates increases with enzyme concentration is well documented. It would therefore seem plausible that for surface bound substrates, such as is encountered in many cleaning applications, the rate of hydrolysis would increase with increasing surface concentration. This has been shown to be the case. (Brode, P.F. III and D. S. Rauch, LANGMUIR, "Subtilisin BPN': Activity on an Immobilized Substrate", Vol. 8, pp. 1325-1329 (1992)). In fact, a linear dependence of rate upon surface concentration was found for insoluble substrates when the surface concentration of the enzyme was varied. (Rubingh, D. N. and M. D. Bauer. "Catalysis of Hydrolysis by Proteases at the Protein-Solution Interface." in POLYMER SOLUTIONS, BLENDS AND INTERFACES. Ed. by I. Noda and D. N. Rubingh. Elsevier, p. 464 (1992)). Surprisingly, when seeking to apply this principle in the search for variant proteases which give better cleaning performance, we did not find that enzymes which adsorb more give better performance. In fact, we surprisingly determined the opposite to be the case: decreased adsorption by an enzyme to a substrate resulted in increased hydrolysis of the substrate (i.e., better cleaning performance).

While not wishing to be bound by theory, it is believed that improved performance, when comparing one variant to another, is a result of the fact that enzymes which adsorb less are also less tightly bound and therefore more highly mobile on the surface from which the insoluble protein substrate is to be removed. At comparable enzyme solution concentrations, this increased mobility is sufficient to outweigh any advantage that is conferred by delivering a higher concentration of enzyme to the surface.

The mutations described herein are designed to change (i.e., decrease) the adsorption of the enzyme to surface-bound soils. In Thermitase, certain amino acids form exterior loops on the enzyme molecule. For purposes of discussion, these loops shall be referred to as first, second, third, fourth and fifth loop regions. Specifically, positions 66-73 form the first loop region; positions 103-115 form the second loop region; positions 134-141 form the third loop region; positions 162-171 form the fourth loop region; positions 191-195 form the fifth loop region; and positions 204-224 form the sixth loop region (position numbering analogous to positions in the amino acid sequence for wild-type subtilisin Thermitase (SEQ ID NO:1)).

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It is believed that these loop regions play a significant role in the adsorption of the enzyme molecule to a surface-bound peptide, and specific mutations in one or more of these loop regions will have a significant effect on this adsorption. While not wishing to be bound by theory, it is believed that the loop regions are important to the adsorption of the Thermitase molecule for at least two reasons. First, the amino acids which comprise the loop regions can make close contacts with any surfaces to which the molecule is exposed. Second, the proximity of the loop regions to the active-site and binding pocket of the Thermitase molecule gives them a role in the catalytically productive adsorption of the enzyme to surface-bound substrates (peptides/protein soils).

As used herein, "variant" means an enzyme having an amino acid sequence which differs from that of wild-type.

As used herein, "mutant Thermitase DNA" means a DNA sequence coding for a Thermitase variant.

As used herein, "wild-type Thermitase" refers to an enzyme represented by SEQ ID NO:1. The amino acid sequence for Thermitase is further described by Meloun, B., Baudys, M., Kostka, V., Hausdorf, G., Frommel, C., and Hohne, W.E., FEBS LETT., Vol. 183, pp. 195-200 (1985), incorporated herein by reference.

As used herein, the term "Thermitase wild-type amino acid sequence" encompasses SEQ ID NO:1 as well as SEQ ID NO:1 having modifications to the amino acid sequence other than at any of positions 66, 67, 68, 69, 70, 72, 73, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 134, 135, 136, 137, 138, 139, 140, 141, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 191, 192,193,194, 195, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224.

As used herein, "more hydrophilic amino acid" refers to any other amino acid having greater hydrophilicity than a subject amino acid with reference to the hydrophilicity table below. The following hydrophilicity table (Table 1) lists amino acids in descending order of increasing hydrophilicity (see Hopp, T.P., and Woods, K.R., "Prediction of Protein Antigenic Determinants from Amino Acid Sequences", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, Vol. 78, pp. 3824-3828, 1981, incorporated herein by reference).

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TABLE 1

Amino Acid	Hydrophilicity Value
Trp	-3.4
Phe	-2.5
Tyr	-2.3
Leu, Ile	-1.8
Val	-1.5
Met	-1.3
Cys	-1.0
Ala, His	-0.5
Thr	-0.4
Pro, Gly	-0.0
Gln, Asn	0.2
Ser	0.3
Arg^+, Lys^+, Glu^-, Asp^-	3.0

Table 1 also indicates which amino acids carry a charge (this characteristic being based on a pH of from about 8-9). The positively charged amino acids are Arg and Lys, the negatively charged amino acids are Glu and Asp, and the remaining amino acids are neutral. In a preferred embodiment of the present invention, the substituting amino acid is either neutral or negatively charged, more preferably negatively charged (i.e., Glu or Asp).

Therefore, for example, the statement "substitute Gln with an equally or more hydrophilic amino acid which is neutral or has a negative charge" means Gln would be substituted with Asn (which is equally hydrophilic to Gln), or Ser, Glu or Asp (which are more hydrophilic than Gln); each of which are neutral or have a negative charge, and have a greater hydrophilicity value as compared to Gln. Likewise, the statement "substitute Pro with a more hydrophilic amino acid which is neutral or has a negative charge" means Pro would be substituted with Gln, Asn, Ser, Glu or Asp.

In one embodiment of the present invention, the Thermitase variant has a modified amino acid sequence of Thermitase wild-type amino acid sequence, wherein the wild-type amino acid sequence comprises a substitution at one or more positions in one or more of the first loop region, the second loop region, the third loop region, the fourth loop region, the fifth loop region or the sixth loop region; whereby the Thermitase variant has

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decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Thermitase.

In a preferred embodiment of the present invention, the substituting amino acid for one or more of the positions in one or more of the loop regions is, with reference to Table 1, neutral or negatively charged and equally or more hydrophilic, preferably more hydrophilic, than the amino acid at the subject position in the wild-type amino acid sequence.

A.   Substitutions in the First Loop Region

When a substitution occurs in the first loop region, the substitution occurs at one or more of positions 66, 67, 68, 69, 70, 72 or 73.

When a substitution occurs at position 66, the substituting amino acid is Asn, Asp, Glu or Ser.

When a substitution occurs at position 67, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 68, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 69, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 70, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 72, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 73, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

B.   Substitutions in the Second Loop Region

When a substitution occurs in the second loop region, the substitution occurs at one or more of positions 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114 or 115.

When a substitution occurs at position 103, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 104, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser , Thr or Val.

When a substitution occurs at position 105, the substituting amino acid is Glu.

When a substitution occurs at position 106, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 107, the substituting amino

acid is Asp or Glu.

When a substitution occurs at position 108, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 109, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 110, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 111, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 112, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 113, the substituting amino acid is Asn, Asp, Gln. Glu, Gly, Pro or Ser.

When a substitution occurs at position 114, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 115, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

C.   Substitutions in the Third Loop Region

When a substitution occurs in the third loop region, the substitution occurs at one or more of positions 134, 135, 136, 137, 138, 139, 140 or 141. wherein

When a substitution occurs at position 134, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 135, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 136, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 137, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 138, the substituting amino acid is Ala, Asn, Asp, cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 139, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 140, the substituting amino acid is Asp, gln, Glu or Ser. and

When a substitution occurs at position 141, the substituting amino

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acid is Asp or Glu.

D.   Substitutions in the Fourth Loop Region

When a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 162, 163, 164, 165, 166, 167, 168, 169, 170 or 171.

When a substitution occurs at position 162, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 163, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 164, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 165, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 166, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 167, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 168, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 169, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 170, the substituting amino acid is Asp, Gln, Glu or Ser. and

When a substitution occurs at position 171, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

E.   Substitutions in the Fifth Loop Region

When a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 191, 192, 193, 194 or 195.

When a substitution occurs at position 191, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 192, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 193, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 194, the substituting amino

acid is Asp or Glu.

When a substitution occurs at position 195, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

F.   Substitutions in the Sixth Loop Region

When a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218 or 219, 220, 221, 223 or 224.

When a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 206, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 207, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 209, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 210, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 211, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 212, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 213, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr or Val.

When a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, or Ser.

When a substitution occurs at position 215, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 216, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 217, the substituting amino

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acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 218, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 219, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 220, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 221, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 222, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 223, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 224, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

G.   Preparation of enzyme variants

Example 1

Mutant Thermitase DNA

A phagemid ("TP") containing the wild type Thermitase gene is constructed. The 2.8 Kbp *Pvu* II restriction enzyme fragment of plasmid pUC119, (Vieira, J. and Messing, J., "Production of Single-Stranded Plasmid DNA", 153 METHODS IN ENZYMOLOGY 3-11 (1989)) is cloned into the *Pvu* II site of plasmid pUB110 (Bacillus Genetic Stock Center, Columbus, OH 1E9). The pUC119-pUB110 hybrid plasmid is named pJMA601. Into the *Bam*H1 restriction site of PJMA601 is cloned the polymerase chain reaction-amplified Thermitase gene gene giving TP. Phagemid TP is transformed into *Escherichia coli* Ung⁻ strain CJ236 and a single stranded uracil-containing DNA template is produced using the VCSM13 helper phage (Kunkel, T.A., J.D. Roberts and R.A. Zakour, "Rapid and efficient site-specific mutagenesis without phenotypic selection", METHODS IN ENZYMOLOGY, Vol. 154, pp. 367-382, (1987); as modified by Yuckenberg, P.D., F. Witney, J. Geisselsoder and J. McClary, "Site-directed in vitro mutagenesis using uracil-containing DNA and phagemid vectors", DIRECTED MUTAGENESIS - A PRACTICAL APPROACH, ed. M.J. McPherson, pp. 27-48, (1991); both of which are incorporated herein by reference). A single primer site-directed mutagenesis modification of the method of Zoller and Smith

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(Zoller, M.J., and M. Smith, "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA", NUCLEIC ACIDS RESEARCH, Vol. 10, pp. 6487-6500, (1982), incorporated herein by reference) is used to produce all mutants (basically as presented by Yuckenberg, et al., 1991, above). Oligonucleotides are made using an Applied Biosystem Inc. 380B DNA synthesizer. Mutagenesis reaction products are transformed into *Escherichia coli* strain MM294 (American Type Culture Collection *E. coli.* 33625). All mutants are confirmed by DNA sequencing and the isolated DNA is transformed into the *Bacillus subtilis* expression strain BG2036 (Yang, M. Y., E. Ferrari and D. J. Henner, (1984), "Cloning of the Neutral Protease Gene of *Bacillus subtilis* and the Use of the Cloned Gene to Create an In Vitro-derived Deletion Mutation", JOURNAL OF BACTERIOLOGY, Vol. 160, pp. 15-21). For some of the mutants a modified TP with a frameshift-stop codon mutation in the corresponding loop is used to produce the uracil template. Oligonucleotides are designed to restore the proper reading frame and to encode for random substitutions at positions 66, 67, 68, 69, 70, 72, 73, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 134, 135, 136, 137, 138, 139, 140, 141, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 191, 192,193,194, 195, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223 or 224 (equimolar and/or variable mixtures of all four nucleotides for all three bases at these codons). Mutations that correct for the frameshift-stop and produce a functional enzyme are identified by their ability to digest casein. The random substitutions are determined by DNA sequencing.

Example 2

Fermentation

The *Bacillus subtilis* cells (BG2036) containing a subtilisin mutant of interest are grown to mid-log phase in a one liter culture of LB-glucose broth and inoculated into a Biostat ED fermenter (B. Braun Biotech, Inc., Allentown, Pennsylvania) in a total volume of 10 liters. The fermentation media contains Yeast Extract, starch, antifoam, buffers and trace minerals (see FERMENTATION: A PRACTICAL APPROACH, Ed. B. McNeil and L. M. Harvey, 1990). The broth is kept at a constant pH of 7.0 during the fermentation run. Chloramphenical is added for antibiotic selection of mutagenized plasmid. The cells are grown overnight at 37°C to an A_{600} of about 60 and harvested.

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Example 3
Purification

The fermentation broth is taken through the following steps to obtain pure enzyme. The broth is cleared of *Bacillus subtilis* cells by centrifugation, and clarified by removing fine particulates with a 100K cutoff membrane. This is followed by concentration on a 10K cutoff membrane, and flow dialysis to reduce the ionic strength and adjust the pH to 5.5 using 0.025M MES buffer (2-(*N*-morpholino)ethanesulfonic acid). The enzyme is further purified by loading it onto either a cation exchange chromatography column or an affinity adsorption chromatography column and eluting it from the column with a NaCl or a propylene glycol gradient (*see* Scopes, R. K., PROTEIN PURIFICATION PRINCIPLES AND PRACTICE, Springer-Verlag, New York (1984), incorporated herein by reference).

The *p*NA assay (DelMar, E.G., C. Largman, J.W. Brodrick and M.C. Geokas, ANAL. BIOCHEM., Vol. 99, pp. 316-320, (1979), incorporated herein by reference) is used to determine the active enzyme concentration for fractions collected during gradient elution. This assay measures the rate at which *p*-nitroaniline is released as the enzyme hydrolyzes the soluble synthetic substrate, succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (sAAPF-*p*NA). The rate of production of yellow color from the hydrolysis reaction is measured at 410 nm on a spectrophotometer and is proportional to the active enzyme concentration. In addition, absorbance measurements at 280 nm are used to determine the total protein concentration. The active enzyme/total-protein ratio gives the enzyme purity, and is used to identify fractions to be pooled for the stock solution.

To avoid autolysis of the enzyme during storage, an equal weight of propylene glycol is added to the pooled fractions obtained from the chromatography column. Upon completion of the purification procedure the purity of the stock enzyme solution is checked with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and the absolute enzyme concentration is determined via an active site titration method using trypsin inhibitor type II-T: turkey egg white purchased from Sigma Chemical Company (St. Louis, Missouri). The measured conversion factors will show which changes made in the enzyme molecule at the various positions result in the enzyme variant having increased activity over the wild-type, against the soluble substrate *p*NA.

In preparation for use, the enzyme stock solution is eluted through a

Sephadex-G25 (Pharmacia, Piscataway, New Jersey) size exclusion column to remove the propylene glycol and exchange the buffer. The MES buffer in the enzyme stock solution is exchanged for 0.1 M Tris buffer (Tris(hydroxymethyl-aminomethane) containing 0.01M $CaCl_2$ and pH adjusted to 8.6 with HCl. All experiments are carried out at pH 8.6 in Tris buffer thermostated at 25°C.

H.   Characterization of enzyme variants

Example 4

Model Surface Preparation

Aminopropyl controlled pore glass (CPG) purchased from CPG Inc. (Fairfield, New Jersey) is used as a support for covalently attaching the sAAPF-pNA substrate purchased from Bachem, Inc. (Torrence, California). The reaction is carried out in dimethyl sulfoxide and (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride) (EDC) is used as a coupling agent. Upon completion (monitored by pNA assay), the excess solvent is removed, and the CPG:sAAPF-pNA is rinsed with dimethyl sulfoxide (DMSO) and doubly-distilled water. This is followed by oven drying with a N_2 purge at about 70°C. The reaction scheme and preparation of the immobilized substrate are conducted as described by Brode, P.F. III, and D.S. Rauch, "Subtilisin BPN': Activity on an Immobilized Substrate," LANGMUIR, Vol. 8, p. 1325-1329, (1992), incorporated herein by reference.

The CPG surface will have $62,000 \pm 7,000$ pNA molecules/μm^2. The surface area will remain unchanged from the value of 50.0m^2/g reported by CPG Inc. for the CPG as received. This suggests that the procedure used to add sAAPF-pNA to CPG does not damage the porous structure (mean diameter is 486 Å).

Example 5

Surface Hydrolysis Assay

Using CPG:sAAPF-pNA, adsorption of an enzyme variant and hydrolysis of a CPG-bound peptide can be measured in a single experiment. A small volume of enzyme variant stock solution is added to a flask containing Tris buffer and CPG:sAAPF-pNA which has been degassed. The flask is shaken on a wrist-action shaker for a period of 90 minutes during which the shaker is stopped at various time intervals (for example, every 2 minutes during the early stages of adsorption hydrolysis - e.g., the first 20 minutes - and every 10 minutes towards the end of the

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experiment). The CPG:sAAPF-pNA is allowed to settle and the solution is sampled. Both the experimental procedure and the calculation of the adsorption and hydrolysis are conducted as described by Brode *et al.*, 1992, above.

All enzymes are monitored for stability against autolysis and should show no appreciable autolytic loss over the time course of this experiment. Therefore, enzyme adsorption can be determined by measuring solution depletion. The difference between the initial enzyme variant concentration and the concentration measured at each individual time point gives the amount of enzyme variant adsorbed. The amount of pNA hydrolyzed from the surface is measured by taking an absorbance reading on an aliquot of the sample at 410 nm. The total amount of pNA hydrolyzed is calculated by adding the amount sampled and the amount remaining in the flask. This value is corrected by subtracting the amount of pNA that is hydrolyzed by Tris buffer at pH 8.6 when no enzyme is present. This base-hydrolysis ranges from 7-29% of the total hydrolysis depending on the efficiency of the enzyme.

Example 6
Soluble Substrate Kinetic Analysis

The rates of hydrolysis of the soluble substrate sAAPF-pNA are monitored by measuring the adsorbance increase as a function of time at 410 nm on a DU-70 spectrophotometer. The enzyme concentration is held constant and is prepared to be in the range of 6-10 nanomolar while the substrate concentration is varied from 90-700 μM sAAPF-pNA for each kinetic determination. An adsorbance data point is taken each second over a period of 900 seconds and the data are transferred to a LOTUS™ spreadsheet (Lotus Development Corporation, Cambridge, Massachusetts). Analysis for kinetic parameters is conducted by the standard Lineweaver Burk analysis in which the data in the initial part of the run (generally the first minute) are fit to a linear regression curve to give v_o. The v_o and s_o data are plotted in the standard inverse fashion to give K_M and k_{cat}.

I.   <u>Example Thermitase variants</u>

Thermitase variants of the present invention which have decreased adsorption to and increased hydrolysis of surface bound substrates are exemplified in Tables 2-36, below. In describing the specific mutations, the original amino acid occurring in wild-type is given first, the position number second, and the substituted amino acid third.

TABLE 2

Loop 1 - Single Mutation Variants
Gln66Asn
Gln66Asp
Gln66Glu
Gln66Ser
Asn67Asp
Asn67Gln
Asn67Glu
Asn67Ser
Gly68Asn
Gly68Asp
Gly68Gln
Gly68Glu
Gly68Pro
Gly68Ser
Asn69Asp
Asn69Gln
Asn69Glu
Asn69Ser
Gly70Asn
Gly70Asp
Gly70Gln
Gly70Glu
Gly70Pro
Gly70Ser
Gly72Asn
Gly72Asp
Gly72Gln
Gly72Glu
Gly72Pro
Gly72Ser
Thr73Asn
Thr73Asp
Thr73Gln
Thr73Glu
Thr73Gly
Thr73Pro
Thr73Ser

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TABLE 3

Loop 1 - Double Mutation Variants

```
Asn68Asp + Gly72Pro
Gly69Asn + Thr73Pro
Asn66Glu + Gly69Ser
Asn66Glu + Gly67Ser
Gly69Gln + Thr73Pro
Gly67Ser + Gly72Ser
Gly67Asp + Thr73Gly
Gly69Pro + Ser70Asp
Gly67Pro + Gly69Pro
Asn66Glu + Thr73Gly
Gly69Gln + Gly72Asp
Asn66Gln + Thr73Gly
Asn68Asp + Thr73Ser
Asn66Gln + Gly69Asn
Gly67Glu + Thr73Ser
Gly72Glu + Thr73Pro
Asn66Glu + Thr73Pro
Gly72Asp + Thr73Gln
Asn68Ser + Gly69Glu
Gly67Pro + Gly69Ser
Gly67Pro + Gly72Asp
Gly69Ser + Gly72Glu
Ser70Asp + Thr73Gln
Gly67Ser + Asn68Glu
Gly69Ser + Thr73Ser
Asn68Asp + Gly69Ser
Asn66Ser + Gly72Asp
Gly69Asp + Gly72Ser
Asn68Ser + Thr73Pro
Ser70Asp + Thr73Ser
Ser70Glu + Gly72Asn
Gly69Asp + Thr73Pro
Gly67Glu + Thr73Gly
Gly72Asp + Thr73Ser
Ser70Asp + Thr73Asn
Asn66Ser + Gly69Glu
Gly72Ser + Thr73Gln
Ser70Glu + Thr73Ser
Asn66Gln + Gly69Ser
```

TABLE 4

Loop 1 - Triple Mutation Variants

```
Ser70Asp + Gly72Gln + Thr73Gly
Gly67Ser + Asn68Gln + Ser70Asp
Asn66Ser + Gly67Asn + Thr73Gln
Asn66Gln + Ser70Asp + Gly72Asn
Ser70Glu + Gly72Gln + Thr73Ser
```

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Gly67Pro + Asn68Glu + Thr73Ser
Asn66Ser + Gly69Gln + Gly72Gln
Asn66Ser + Ser70Asp + Thr73Gln
Gly67Gln + Gly69Asp + Thr73Gln
Gly69Pro + Gly72Glu + Thr73Ser
Gly67Gln + Gly69Asn + Thr73Asn
Asn66Ser + Gly69Asp + Thr73Asn
Gly69Asp + Gly72Ser + Thr73Ser
Asn68Asp + Gly72Gln + Thr73Ser
Asn66Gln + Asn68Ser + Thr73Asn
Asn68Ser + Gly69Ser + Ser70Asp
Asn68Gln + Gly69Ser + Thr73Gln
Gly67Ser + Gly69Pro + Gly72Glu
Gly67Asn + Gly69Ser + Thr73Asn
Gly67Gln + Gly72Asn + Thr73Gln
Gly67Pro + Asn68Gln + Gly69Pro
Gly69Asn + Ser70Glu + Thr73Ser
Asn66Ser + Ser70Glu + Gly72Asn
Gly69Gln + Ser70Asp + Gly72Asn
Gly67Ser + Ser70Glu + Thr73Gln
Asn66Glu + Gly67Gln + Thr73Pro
Gly67Ser + Gly72Glu + Thr73Ser
Gly67Pro + Gly69Gln + Gly72Asp
Asn66Ser + Gly67Glu + Thr73Pro
Asn66Ser + Gly67Glu + Asn68Ser
Gly67Asn + Asn68Glu + Gly69Asp
Asn68Glu + Gly69Glu + Gly72Pro
Asn68Glu + Gly69Glu + Thr73Asn
Gly67Glu + Asn68Asp + Gly69Gln
Gly67Glu + Asn68Asp + Thr73Gln
Asn66Asp + Gly67Asp + Gly72Ser
Asn66Glu + Gly67Glu + Thr73Pro

TABLE 5

Loop 1 - Quadruple Mutation Variants

Asn66Gln + Gly67Ser + Gly69Asp + Gly72Pro
Asn68Ser + Ser70Glu + Gly72Gln + Thr73Pro
Asn66Gln + Gly67Gln + Ser70Asp + Thr73Asn
Gly67Ser + Gly69Asp + Gly72Gln + Thr73Gly
Asn66Glu + Gly67Ser + Asn68Ser + Thr73Asn
Gly67Ser + Asn68Asp + Gly72Asn + Thr73Gly
Asn66Ser + Gly67Glu + Gly69Gln + Thr73Gly
Asn68Glu + Gly69Asp + Gly72Asn + Thr73Asn
Gly67Glu + Asn68Glu + Gly72Asn + Thr73Asn
Asn66Glu + Gly67Glu + Asn68Gln + Gly72Pro
Asn66Glu + Gly67Glu + Asn68Ser + Gly72Asn
Asn66Glu + Gly67Glu + Gly72Pro + Thr73Asn
Gly67Ser + Gly69Asp + Ser70Glu + Thr73Gln
Asn66Ser + Gly69Glu + Ser70Glu + Thr73Gly
Gly67Pro + Asn68Glu + Gly69Glu + Ser70Glu
Gly67Ser + Asn68Glu + Gly69Asp + Ser70Asp

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```
Gly67Asn + Asn68Asp + Gly69Asp + Ser70Glu
Gly67Asp + Asn68Gln + Gly69Glu + Ser70Asp
Asn66Gln + Gly67Asp + Gly69Asp + Ser70Asp
Gly67Asp + Asn68Asp + Ser70Asp + Thr73Asn
Gly67Asp + Asn68Asp + Gly69Pro + Ser70Glu
Gly67Asp + Asn68Gln + Ser70Glu + Gly72Pro
Asn66Gln + Gly67Glu + Ser70Asp + Gly72Gln
Gly67Glu + Ser70Glu + Gly72Gln + Thr73Ser
Gly67Asp + Asn68Gln + Ser70Asp + Gly72Ser
Gly67Glu + Ser70Asp + Gly72Ser + Thr73Ser
```

TABLE 6

Loop 2 - Single Mutation Variants
Val103Ala
Val103Asn
Val103Asp
Val103Cys
Val103Gln
Val103Glu
Val103Gly
Val103His
Val103Met
Val103Pro
Val103Ser
Val103Thr
Leu104Ala
Leu104Asn
Leu104Asp
Leu104Cys
Leu104Gln
Leu104Glu
Leu104Gly
Leu104His
Leu104Ile
Leu104Met
Leu104Pro
Leu104Ser
Leu104Thr
Leu104Val
Asp105Glu
Asn106Asp
Asn106Gln
Asn106Glu
Asn106Ser
Ser107Asp
Ser107Glu
Gly108Asn
Gly108Asp
Gly108Gln
Gly108Glu
Gly108Pro

```
Gly108Ser
Ser109Asp
Ser109Glu
Gly110Asn
Gly110Asp
Gly110Gln
Gly110Glu
Gly110Pro
Gly110Ser
Thr111Asn
Thr111Asp
Thr111Gln
Thr111Glu
Thr111Gly
Thr111Pro
Thr111Ser
Trp112Ala
Trp112Asn
Trp112Asp
Trp112Cys
Trp112Gln
Trp112Glu
Trp112Gly
Trp112His
Trp112Ile
Trp112Leu
Trp112Met
Trp112Phe
Trp112Pro
Trp112Ser
Trp112Thr
Trp112Tyr
Trp112Val
Thr113Asn
Thr113Asp
Thr113Gln
Thr113Glu
Thr113Gly
Thr113Pro
Thr113Ser
Ala114Asn
Ala114Asp
Ala114Gln
Ala114Glu
Ala114Gly
Ala114His
Ala114Pro
Ala114Ser
Ala114Thr
Val115Ala
Val115Asn
Val115Asp
Val115Cys
```

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```
            Val115Gln
            Val115Glu
            Val115Gly
            Val115His
            Val115Met
            Val115Pro
            Val115Ser
            Val115Thr
```

TABLE 7

Loop 2 - Double Mutation Variants
Val103Gln + Ser109Glu
Asp105Glu + Gly108Gln
Asp105Glu + Gly110Asn
Asp105Glu + Gly110Pro
Val103Asp + Thr111Gln
Leu104Gln + Gly108Asp
Leu104Gly + Ser107Glu
Thr111Pro + Ala114Ser
Asn106Gln + Ser109Asp
Asp105Glu + Gly108Pro
Thr113Asp + Val115Gly
Val103Met + Leu104Gln
Gly108Gln + Thr113Asn
Gly110Glu + Ala114Gly
Thr111Gln + Val115Gln
Gly110Glu + Thr111Gln
Gly108Pro + Ala114Glu
Gly108Ser + Val115Gly
Asp105Glu + Thr113Gln
Leu104His + Ser107Asp
Asp105Glu + Ala114Gln
Gly110Gln + Thr113Ser
Val103His + Ala114Gln
Asp105Glu + Trp112Ile
Thr111Ser + Thr113Asp
Leu104His + Thr111Glu
Asn106Gln + Trp112Tyr
Leu104Gly + Thr113Gln
Val103Thr + Val115Asn
Val103Thr + Asn106Ser
Val103Pro + Thr111Pro
Ser109Glu + Val115Met
Val103Ala + Asn106Gln
Val103Cys + Thr111Gln
Trp112Gly + Thr113Gly
Val103Gly + Asp105Glu
Gly110Asn + Thr113Gly
Thr111Asn + Thr113Glu
Val103Glu + Trp112Met
Leu104Pro + Ser109Asp

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```
Gly108Gln + Ala114Gly
Leu104Met + Val115Glu
Leu104Gly + Ala114Ser
Thr113Ser + Val115Ala
Asp105Glu + Asn106Gln
Val103Thr + Asp105Glu
Gly110Glu + Val115Met
Val103Gly + Gly110Glu
Ser109Asp + Gly110Gln
Thr111Asp + Ala114Ser
Asn106Glu + Ala114Pro
Ser109Glu + Thr113Gly
Gly108Asn + Trp112Glu
Val103Pro + Asn106Glu
Asp105Glu + Trp112Leu
Leu104Cys + Asp105Glu
Leu104Cys + Thr111Ser
Leu104Thr + Ser109Asp
Val103Asn + Val115Glu
Val103Ser + Gly108Glu
```

TABLE 8

Loop 2 - Triple Mutation Variants

```
Val103Gln + Leu104Thr + Ser109Glu
Leu104Ser + Asp105Glu + Trp112Phe
Asn106Gln + Gly108Ser + Gly110Gln
Ser109Asp + Thr111Asn + Trp112Thr
Asn106Ser + Gly108Gln + Ser109Asp
Asp105Glu + Gly108Pro + Val115Gly
Asn106Ser + Ser107Asp + Val115Gly
Asn106Ser + Trp112Gln + Val115Glu
Leu104Pro + Ser109Asp + Thr113Gly
Leu104Asp + Gly110Gln + Val115Pro
Gly108Asn + Thr111Ser + Ala114Pro
Leu104Ser + Asn106Ser + Thr111Glu
Val103His + Leu104Gln + Asn106Asp
Asn106Gln + Trp112Asn + Ala114Asp
Val103Met + Gly110Pro + Thr111Pro
Leu104Thr + Gly108Glu + Gly110Asn
Leu104Ala + Ser109Asp + Trp112Cys
Gly108Glu + Gly110Pro + Val115Gly
Leu104Pro + Thr113Asn + Ala114Asp
Val103Asn + Leu104Met + Asn106Glu
Val103Cys + Ser109Glu + Val115Cys
Val103Gly + Leu104Val + Ser107Asp
Gly108Pro + Ser109Glu + Trp112Gln
Leu104Met + Trp112Cys + Thr113Asn
Asn106Asp + Trp112Ile + Ala114Ser
Val103Gly + Ser109Glu + Ala114Pro
Leu104Ile + Trp112Leu + Thr113Gly
Asn106Gln + Gly108Asn + Ser109Glu
```

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```
Val103Asn  +  Trp112Pro  +  Ala114Asp
Gly108Asn  +  Gly110Ser  +  Trp112Gly
Gly110Asp  +  Trp112Leu  +  Val115His
Val103Gly  +  Leu104Gly  +  Gly108Asp
Leu104Ala  +  Thr111Ser  +  Val115Ala
Thr111Ser  +  Thr113Asn  +  Val115His
Asp105Glu  +  Gly108Gln  +  Val115Gly
Leu104Gly  +  Ala114Ser  +  Val115Ala
Val103His  +  Leu104Gly  +  Asn106Glu
Val103Ser  +  Gly110Glu  +  Thr113Pro
Val103Pro  +  Gly110Asn  +  Ala114Glu
Leu104Met  +  Gly110Asp  +  Val115Pro
Leu104Asp  +  Thr111Gln  +  Trp112Ala
Ser109Asp  +  Gly110Pro  +  Trp112Asn
Leu104Asp  +  Asp105Glu  +  Trp112Cys
Gly108Glu  +  Ser109Glu  +  Ala114Thr
Gly108Asp  +  Ser109Asp  +  Thr111Pro
Gly108Glu  +  Ser109Glu  +  Ala114Asn
Asn106Ser  +  Ser109Glu  +  Gly110Asp
Asn106Gln  +  Ser109Asp  +  Gly110Asp
Val103Asp  +  Leu104Asp  +  Gly108Pro
Leu104Thr  +  Asp105Glu  +  Asn106Glu
Asp105Glu  +  Asn106Asp  +  Thr113Asn
Gly108Gln  +  Thr113Glu  +  Ala114Glu
Leu104Ser  +  Thr111Glu  +  Trp112Glu
Asn106Asp  +  Ser107Asp  +  Gly108Asp
Asp105Glu  +  Ser109Asp  +  Gly110Glu
Trp112Ala  +  Thr113Asp  +  Val115Asp
Val103Gln  +  Asp105Glu  +  Gly108Asp
Leu104Gln  +  Asp105Glu  +  Ser107Glu
Ser107Asp  +  Ser109Asp  +  Thr111Gly
Val103Cys  +  Ser107Asp  +  Ser109Glu
```

TABLE 9

Loop 2 - Quadruple Mutation Variants

```
Asp105Glu  +  Gly110Ser  +  Trp112Phe  +  Thr113Ser
Val103Ala  +  Ser107Asp  +  Gly110Pro  +  Ala114Asn
Val103Gln  +  Gly108Pro  +  Thr111Ser  +  Ala114Glu
Val103Thr  +  Ser107Asp  +  Thr113Gln  +  Val115Cys
Leu104Asn  +  Gly110Ser  +  Thr113Glu  +  Val115Gln
Val103Pro  +  Gly108Glu  +  Trp112His  +  Val115Met
Val103Ala  +  Asn106Ser  +  Gly108Asp  +  Thr111Asn
Val103Ala  +  Leu104Asp  +  Gly110Ser  +  Val115Ala
Ser109Glu  +  Trp112Pro  +  Ala114Asn  +  Val115Met
Val103Thr  +  Asn106Asp  +  Gly108Ser  +  Gly110Gln
Val103Pro  +  Leu104Pro  +  Asn106Ser  +  Gly110Asn
Ser109Glu  +  Gly110Pro  +  Thr111Ser  +  Thr113Ser
Asn106Gln  +  Gly108Ser  +  Trp112Tyr  +  Ala114Thr
Leu104Cys  +  Gly110Gln  +  Thr111Asp  +  Thr113Gly
Leu104Ser  +  Asp105Glu  +  Gly108Gln  +  Val115Asn
Val103Asp  +  Gly108Asn  +  Thr113Pro  +  Ala114Pro
```

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```
Val103Asp + Leu104Asn + Thr113Asn + Val115Ala
Val103Asn + Gly110Asp + Thr111Asn + Val115Cys
Val103Ser + Asn106Glu + Gly110Gln + Val115Met
Leu104Val + Thr111Gln + Trp112His + Ala114Ser
Leu104Ser + Asp105Glu + Gly110Gln + Thr111Asn
Val103Ala + Asp105Glu + Asn106Ser + Gly110Asn
Val103Glu + Gly110Ser + Trp112Phe + Val115Gly
Asn106Glu + Thr113Asn + Ala114Pro + Val115Asn
Leu104Glu + Asp105Glu + Gly108Ser + Gly110Asn
Gly108Glu + Ser109Glu + Thr111Asn + Ala114Pro
Gly108Glu + Ser109Glu + Thr111Gln + Ala114Thr
Leu104Ile + Gly108Pro + Ser109Glu + Gly110Asp
Val103Glu + Leu104Asp + Trp112Cys + Thr113Ser
Asp105Glu + Asn106Glu + Gly110Pro + Thr113Gly
Gly110Gln + Thr111Glu + Trp112Glu + Thr113Gly
Ser107Asp + Gly108Asp + Ser109Asp + Trp112Val
Asn106Asp + Ser107Asp + Gly108Glu + Val115Pro
Asp105Glu + Ser107Glu + Gly108Asp + Gly110Ser
Asp105Glu + Gly108Asp + Ser109Glu + Thr111Gln
Asp105Glu + Gly108Glu + Ser109Glu + Thr111Pro
Leu104Asp + Ser109Glu + Trp112Asn + Ala114Gly
Val103Ser + Leu104Glu + Gly108Asn + Ser109Glu
Asp105Glu + Ser109Glu + Gly110Glu + Trp112Gln
Val103Ser + Asp105Glu + Gly108Glu + Val115Pro
Asp105Glu + Ser107Asp + Trp112Leu + Thr113Gly
Leu104His + Asp105Glu + Asn106Ser + Ser107Asp
Asp105Glu + Ser107Glu + Ser109Asp + Val115Gln
Asp105Glu + Ser107Glu + Ser109Glu + Thr113Gly
Ser107Glu + Ser109Glu + Thr111Gln + Thr113Gln
Ser107Glu + Ser109Glu + Trp112Val + Val115Pro
Ser107Glu + Ser109Glu + Trp112Ile + Val115Gln
Asp105Glu + Ser109Glu + Gly110Gln + Val115Pro
Leu104Glu + Asp105Glu + Ser107Glu + Ala114Gly
Asp105Glu + Asn106Glu + Gly108Pro + Ser109Asp
Leu104Asn + Asp105Glu + Gly110Asp + Ala114Gly
Ser107Glu + Gly108Gln + Ser109Asp + Gly110Asp
Gly108Gln + Gly110Glu + Trp112Asp + Val115Glu
Val103Asp + Leu104Ser + Asp105Glu + Val115Gln
Leu104Glu + Asn106Glu + Gly110Glu + Val115Asn
Leu104Asp + Gly110Glu + Thr111Ser + Trp112Asp
Ser109Asp + Gly110Glu + Thr113Asn + Val115Glu
Val103Ser + Gly108Asp + Gly110Glu + Thr111Glu
Leu104Gly + Asn106Glu + Gly108Asn + Ser109Glu
Val103Asp + Leu104Gly + Thr111Glu + Val115Met
```

TABLE 10

Loop 3 - Single Mutation Variants

```
Leu134Ala
Leu134Asn
Leu134Asp
Leu134Cys
```

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```
Leu134Gln
Leu134Glu
Leu134Gly
Leu134His
Leu134Ile
Leu134Met
Leu134Pro
Leu134Ser
Leu134Thr
Leu134Val
Gly135Asn
Gly135Asp
Gly135Gln
Gly135Glu
Gly135Pro
Gly135Ser
Gly136Asn
Gly136Asp
Gly136Gln
Gly136Glu
Gly136Pro
Gly136Ser
Thr137Asn
Thr137Asp
Thr137Gln
Thr137Glu
Thr137Gly
Thr137Pro
Thr137Ser
Val138Ala
Val138Asn
Val138Asp
Val138Cys
Val138Gln
Val138Glu
Val138Gly
Val138His
Val138Met
Val138Pro
Val138Ser
Val138Thr
Gly139Asn
Gly139Asp
Gly139Gln
Gly139Glu
Gly139Pro
Gly139Ser
Asn140Asp
Asn140Gln
Asn140Glu
Asn140Ser
Ser141Asp
Ser141Glu
```

TABLE 11

Loop 3 - Double Mutation Variants
Leu134Val + Ser141Asp
Leu134Val + Thr137Glu
Gly135Asn + Ser141Asp
Gly135Ser + Thr137Ser
Leu134Met + Gly139Asp
Leu134Thr + Gly139Gln
Gly139Gln + Asn140Glu
Gly139Pro + Ser141Asp
Leu134Glu + Val138Asn
Leu134Asn + Val138Ala
Leu134Ser + Asn140Glu
Val138Asn + Ser141Asp
Gly135Glu + Val138Ser
Leu134Ile + Gly139Glu
Leu134Ile + Gly135Asn
Leu134Met + Asn140Asp
Gly136Ser + Gly139Asp
Gly136Pro + Val138Asn
Leu134Gly + Gly135Pro
Leu134Gly + Ser141Glu
Gly135Pro + Gly139Pro
Leu134Thr + Gly139Pro
Gly135Glu + Asn140Ser
Thr137Gln + Gly139Asp
Gly135Asp + Asn140Ser
Leu134Ser + Asn140Asp
Gly136Gln + Gly139Asp
Leu134Pro + Ser141Glu
Leu134His + Asn140Gln
Leu134Pro + Gly135Glu
Gly135Pro + Gly136Asp
Val138Thr + Ser141Asp
Val138Pro + Ser141Asp
Leu134Gln + Thr137Glu
Val138Ser + Ser141Asp
Val138Thr + Asn140Gln
Leu134Glu + Val138Gln
Gly139Asn + Ser141Glu
Gly139Pro + Ser141Glu
Gly135Gln + Gly139Glu
Thr137Pro + Val138Gly
Gly135Asp + Gly136Gln
Gly135Asn + Val138Thr
Thr137Asn + Ser141Glu
Gly135Pro + Val138Glu
Gly135Glu + Thr137Ser
Gly135Asp + Gly139Pro
Leu134Ala + Gly135Gln

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```
Val138Gln  +  Ser141Asp
Gly135Ser  +  Gly139Asp
Thr137Ser  +  Val138Met
Leu134Ala  +  Gly136Gln
Leu134Glu  +  Asn140Gln
Gly136Asn  +  Asn140Gln
Gly136Gln  +  Ser141Asp
Val138Ser  +  Ser141Glu
Leu134Thr  +  Thr137Glu
Gly135Ser  +  Gly139Pro
Val138Cys  +  Gly139Asp
Thr137Gly  +  Gly139Asp
```

TABLE 12

Loop 3 - Triple Mutation Variants

```
Gly135Ser  +  Val138Cys  +  Ser141Asp
Gly135Gln  +  Thr137Glu  +  Val138Cys
Leu134Ser  +  Gly135Pro  +  Gly136Glu
Leu134Pro  +  Val138Cys  +  Asn140Ser
Leu134Val  +  Gly135Asp  +  Val138Met
Leu134Pro  +  Gly135Pro  +  Gly139Glu
Gly135Gln  +  Val138Asp  +  Gly139Ser
Gly136Pro  +  Val138His  +  Ser141Asp
Leu134Val  +  Gly136Ser  +  Asn140Asp
Thr137Asp  +  Val138Met  +  Gly139Gln
Leu134Val  +  Gly136Pro  +  Thr137Gly
Gly135Gln  +  Val138Gln  +  Asn140Asp
Leu134His  +  Gly136Glu  +  Asn140Gln
Gly135Pro  +  Gly139Gln  +  Ser141Asp
Gly136Asn  +  Val138Asn  +  Ser141Asp
Gly135Asp  +  Val138Thr  +  Gly139Gln
Gly136Pro  +  Thr137Asp  +  Gly139Ser
Leu134Gly  +  Gly136Gln  +  Gly139Ser
Leu134Val  +  Gly135Glu  +  Thr137Gly
Leu134Thr  +  Val138Gln  +  Gly139Ser
Gly136Asp  +  Thr137Asn  +  Val138Gly
Gly135Asp  +  Val138Ser  +  Asn140Gln
Gly136Asn  +  Val138Glu  +  Asn140Gln
Leu134Cys  +  Thr137Glu  +  Val138Gly
Leu134Asn  +  Gly135Glu  +  Gly139Ser
Gly135Ser  +  Val138His  +  Asn140Glu
Leu134Ala  +  Gly139Ser  +  Asn140Gln
Gly135Asp  +  Thr137Pro  +  Val138Cys
Leu134Cys  +  Val138Gly  +  Asn140Asp
Leu134Thr  +  Val138Ser  +  Gly139Asp
Leu134Cys  +  Thr137Gln  +  Ser141Glu
Leu134Val  +  Gly136Pro  +  Gly139Glu
Leu134Ala  +  Gly135Asn  +  Ser141Glu
Gly136Gln  +  Thr137Gln  +  Val138Pro
Leu134Asp  +  Gly135Pro  +  Gly139Ser
Gly135Ser  +  Val138Gly  +  Ser141Asp
```

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```
Leu134Val + Thr137Asp + Gly139Gln
Leu134His + Gly135Pro + Gly139Gln
Thr137Pro + Val138Gln + Asn140Asp
Thr137Gln + Val138Cys + Gly139Glu
Thr137Ser + Gly139Glu + Asn140Gln
Gly135Gln + Thr137Gln + Asn140Asp
Gly135Gln + Thr137Gln + Val138Asp
Leu134His + Gly139Gln + Asn140Asp
Leu134Ala + Thr137Asn + Gly139Glu
Val138Pro + Gly139Pro + Asn140Gln
Gly135Asn + Asn140Ser + Ser141Glu
Leu134Ile + Gly135Asp + Val138Cys
Thr137Gln + Gly139Ser + Asn140Ser
Leu134Ala + Gly136Pro + Asn140Asp
Gly135Ser + Gly139Asp + Asn140Ser
Leu134Thr + Gly135Asn + Ser141Glu
Leu134Gly + Thr137Ser + Val138Ala
Leu134Thr + Gly135Gln + Val138Pro
Gly135Gln + Gly136Asp + Thr137Ser
Gly135Ser + Val138Thr + Asn140Asp
Leu134Cys + Thr137Ser + Val138Glu
Gly136Ser + Thr137Asn + Ser141Asp
Leu134Thr + Gly135Glu + Gly139Gln
Leu134Thr + Gly135Asp + Thr137Ser
```

TABLE 13

Loop 3 - Quadruple Mutation Variants
Leu134Met + Thr137Pro + Asn140Ser + Ser141Glu
Leu134Asn + Gly136Pro + Thr137Ser + Val138Gln
Leu134Gln + Gly136Gln + Val138Ser + Gly139Ser
Gly135Glu + Gly136Asn + Thr137Pro + Asn140Gln
Leu134Ser + Gly136Asn + Gly139Gln + Ser141Glu
Leu134Gln + Val138Pro + Gly139Ser + Ser141Asp
Leu134Pro + Gly135Asn + Thr137Glu + Asn140Gln
Gly135Asp + Thr137Gly + Val138Gln + Gly139Ser
Leu134Val + Gly135Asp + Thr137Asn + Asn140Ser
Leu134Thr + Gly135Glu + Gly136Ser + Thr137Ser
Gly136Pro + Thr137Gln + Gly139Gln + Asn140Glu
Leu134His + Gly135Glu + Val138Asn + Asn140Gln
Leu134Gln + Gly135Ser + Thr137Asn + Ser141Glu
Gly135Gln + Gly139Asn + Asn140Gln + Ser141Asp
Thr137Gln + Val138His + Gly139Asn + Ser141Glu
Leu134Thr + Gly135Gln + Val138Thr + Asn140Asp
Leu134Met + Gly135Glu + Thr137Ser + Val138Ser
Val138Thr + Gly139Ser + Asn140Ser + Ser141Glu
Leu134Met + Gly136Pro + Val138Cys + Gly139Pro
Leu134His + Gly136Pro + Thr137Gln + Gly139Glu
Gly135Asn + Gly136Gln + Val138Cys + Ser141Glu
Leu134Asn + Thr137Ser + Val138Gln + Ser141Asp
Gly135Gln + Gly136Pro + Thr137Asn + Asn140Ser
Gly135Asn + Gly139Ser + Asn140Gln + Ser141Asp

28

```
Leu134Met + Gly136Pro + Val138Met + Ser141Glu
Leu134Cys + Thr137Gln + Val138Pro + Ser141Asp
Gly136Asn + Thr137Gln + Gly139Asn + Asn140Ser
Leu134Asn + Thr137Ser + Gly139Ser + Asn140Asp
Gly135Asp + Gly136Asp + Val138Met + Asn140Ser
Gly135Glu + Gly136Asp + Val138His + Gly139Gln
Leu134Ser + Gly136Glu + Thr137Glu + Val138Thr
Leu134Thr + Val138Gln + Asn140Glu + Ser141Glu
Gly135Gln + Thr137Asn + Asn140Glu + Ser141Glu
Gly135Gln + Thr137Gly + Val138Asp + Gly139Asp
Leu134Thr + Gly135Glu + Gly136Asp + Thr137Asp
Val138Asn + Gly139Asp + Asn140Glu + Ser141Asp
Leu134Ile + Gly139Glu + Asn140Asp + Ser141Asp
Gly136Pro + Gly139Glu + Asn140Glu + Ser141Asp
Leu134Gln + Gly139Asp + Asn140Asp + Ser141Glu
Leu134Val + Gly136Glu + Val138Asp + Asn140Gln
Gly136Asp + Thr137Gln + Val138Asp + Gly139Asn
Gly136Glu + Thr137Glu + Val138His + Gly139Asp
Leu134His + Thr137Gln + Val138Glu + Asn140Glu
Leu134Gly + Gly135Ser + Thr137Glu + Gly139Glu
Leu134Gln + Gly135Pro + Thr137Asp + Gly139Asp
Leu134His + Gly135Asn + Thr137Asp + Gly139Glu
Leu134Val + Gly135Glu + Thr137Asp + Gly139Gln
Gly135Asn + Thr137Ser + Gly139Asp + Ser141Asp
Leu134Cys + Thr137Asn + Gly139Glu + Ser141Asp
Leu134Ile + Thr137Pro + Gly139Asp + Ser141Asp
Leu134Thr + Val138Asn + Gly139Glu + Ser141Asp
Gly135Pro + Thr137Gly + Gly139Glu + Ser141Asp
Gly136Ser + Val138Ala + Gly139Glu + Ser141Glu
Leu134Thr + Gly139Asp + Asn140Ser + Ser141Asp
Gly135Ser + Thr137Gly + Gly139Glu + Ser141Asp
Leu134Met + Gly135Asn + Gly139Asp + Ser141Glu
Thr137Pro + Val138Gly + Gly139Asp + Ser141Asp
Gly136Glu + Thr137Pro + Val138Gln + Gly139Asp
Thr137Asn + Val138Glu + Gly139Asp + Ser141Asp
Leu134Thr + Val138Glu + Gly139Glu + Ser141Glu
```

TABLE 14

Loop 4 - Single Mutation Variants
Gly162Asn
Gly162Asp
Gly162Gln
Gly162Glu
Gly162Pro
Gly162Ser
Asn163Asp
Asn163Gln
Asn163Glu
Asn163Ser
Ala164Asn
Ala164Asp

Ala164Gln
Ala164Glu
Ala164Gly
Ala164His
Ala164Pro
Ala164Ser
Ala164Thr
Gly165Asn
Gly165Asp
Gly165Gln
Gly165Glu
Gly165Pro
Gly165Ser
Asn166Asp
Asn166Gln
Asn166Glu
Asn166Ser
Thr167Asn
Thr167Asp
Thr167Gln
Thr167Glu
Thr167Gly
Thr167Pro
Thr167Ser
Ala168Asn
Ala168Asp
Ala168Gln
Ala168Glu
Ala168Gly
Ala168His
Ala168Pro
Ala168Ser
Ala168Thr
Pro169Asn
Pro169Asp
Pro169Gln
Pro169Glu
Pro169Gly
Pro169Ser
Asn170Asp
Asn170Gln
Asn170Glu
Asn170Ser
Tyr171Ala
Tyr171Asn
Tyr171Asp
Tyr171Cys
Tyr171Gln
Tyr171Glu
Tyr171Gly
Tyr171His
Tyr171Ile
Tyr171Leu

30

```
Tyr171Met
Tyr171Pro
Tyr171Ser
Tyr171Thr
Tyr171Val
```

TABLE 15

Loop 4 - Double Mutation Variants
Ala164Gln + Pro169Glu
Asn163Asp + Thr167Ser
Ala164Pro + Ala168Asp
Ala164Gln + Asn166Ser
Gly165Asp + Thr167Pro
Ala164Gln + Ala168Ser
Gly165Gln + Asn166Gln
Gly162Gln + Asn163Glu
Gly165Gln + Thr167Glu
Gly165Ser + Ala168Gln
Ala164Gln + Ala168Glu
Ala164His + Tyr171Met
Gly165Pro + Pro169Gln
Gly165Gln + Ala168Pro
Gly162Pro + Ala164Gly
Thr167Pro + Asn170Ser
Asn163Gln + Tyr171His
Asn163Ser + Ala164Gln
Ala164Asp + Asn166Gln
Gly165Asn + Tyr171Ile
Ala168Asp + Asn170Gln
Asn166Asp + Ala168Gly
Asn163Glu + Thr167Ser
Asn166Glu + Ala168Ser
Gly162Gln + Ala168Glu
Gly162Asn + Asn170Ser
Asn163Gln + Ala168His
Thr167Pro + Ala168Thr
Gly162Asn + Gly165Glu
Asn166Glu + Tyr171Pro
Ala168Asn + Asn170Gln
Gly162Glu + Thr167Ser
Thr167Asp + Tyr171Val
Gly162Asp + Ala168His
Gly165Ser + Ala168Glu
Thr167Asp + Tyr171Met
Ala168Thr + Pro169Gly
Gly165Ser + Pro169Glu
Gly162Glu + Ala164Thr
Ala168Ser + Tyr171Asp
Asn163Ser + Gly165Pro
Ala168Asn + Tyr171Ser
Ala164Gln + Gly165Gln

```
Thr167Asp  +  Asn170Ser
Gly165Ser  +  Asn170Gln
Thr167Gly  +  Tyr171Glu
Gly162Asn  +  Tyr171Gln
Gly165Asn  +  Asn166Glu
Ala168Thr  +  Tyr171Glu
Gly165Ser  +  Asn166Glu
Thr167Gly  +  Ala168Thr
Asn166Asp  +  Ala168Asn
Pro169Glu  +  Tyr171Val
Gly162Glu  +  Asn170Ser
Gly162Ser  +  Pro169Gly
Ala164Asn  +  Asn166Glu
Thr167Gly  +  Asn170Asp
Thr167Glu  +  Asn170Ser
Asn163Asp  +  Ala164Gln
Gly165Gln  +  Pro169Glu
```

TABLE 16

Loop 4 - Triple Mutation Variants		
Ala164Gly +	Gly165Glu +	Thr167Ser
Gly162Asn +	Ala164Thr +	Asn166Asp
Ala164Asn +	Gly165Asp +	Pro169Gln
Gly165Pro +	Thr167Asn +	Pro169Glu
Ala168Gly +	Pro169Gly +	Asn170Asp
Gly162Asp +	Gly165Pro +	Ala168Ser
Gly165Asp +	Thr167Asn +	Tyr171Gly
Thr167Ser +	Pro169Asn +	Tyr171Thr
Asn166Ser +	Ala168Gly +	Tyr171Ile
Gly165Gln +	Thr167Pro +	Ala168Glu
Gly162Ser +	Asn163Asp +	Gly165Gln
Gly165Glu +	Thr167Gly +	Ala168Ser
Thr167Gly +	Ala168Asp +	Tyr171Val
Asn163Ser +	Gly165Gln +	Ala168Asp
Asn163Asp +	Ala164Gly +	Gly165Asn
Ala164Gln +	Pro169Ser +	Asn170Gln
Ala164His +	Thr167Gln +	Pro169Gly
Thr167Ser +	Ala168Asn +	Tyr171Asn
Gly162Asp +	Gly165Ser +	Ala168Asn
Gly162Pro +	Asn163Gln +	Tyr171Val
Asn166Glu +	Ala168Ser +	Asn170Gln
Thr167Pro +	Pro169Ser +	Tyr171His
Gly162Gln +	Asn163Gln +	Gly165Asp
Ala164Ser +	Thr167Ser +	Ala168Glu
Thr167Gln +	Pro169Glu +	Asn170Gln
Asn163Gln +	Pro169Asn +	Asn170Gln
Gly162Glu +	Ala168Gln +	Asn170Gln
Ala164Gly +	Gly165Ser +	Asn166Asp
Ala164Ser +	Ala168Gly +	Asn170Gln
Asn163Glu +	Ala164Asn +	Ala168Ser
Thr167Ser +	Pro169Glu +	Tyr171Ser

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```
Ala168Glu  +  Pro169Gln  +  Tyr171Ile
Gly162Pro  +  Ala164Pro  +  Tyr171Pro
Gly162Pro  +  Asn166Ser  +  Pro169Glu
Ala164Gly  +  Ala168Asn  +  Tyr171Ser
Ala164Thr  +  Asn170Asp  +  Tyr171Leu
Asn163Gln  +  Thr167Glu  +  Pro169Ser
Asn166Asp  +  Ala168Gln  +  Tyr171Pro
Gly162Asn  +  Ala164Glu  +  Tyr171Ala
Asn166Glu  +  Thr167Gln  +  Ala168Pro
Ala164Pro  +  Ala168Gln  +  Asn170Asp
Ala164Thr  +  Gly165Gln  +  Ala168Asp
Ala164Ser  +  Ala168Gly  +  Asn170Glu
Ala164His  +  Gly165Glu  +  Tyr171Met
Ala164Gln  +  Gly165Asp  +  Asn166Gln
Gly162Gln  +  Thr167Asn  +  Ala168Asn
Ala168His  +  Pro169Gln  +  Tyr171Glu
Ala164Gln  +  Asn166Gln  +  Tyr171Val
Gly162Pro  +  Ala164Ser  +  Asn170Gln
Thr167Glu  +  Ala168Gly  +  Asn170Ser
Thr167Ser  +  Pro169Gly  +  Asn170Ser
Asn163Glu  +  Asn166Ser  +  Ala168Ser
Asn163Gln  +  Ala168Glu  +  Tyr171Ala
Thr167Ser  +  Ala168Ser  +  Asn170Asp
Gly165Asp  +  Asn166Glu  +  Tyr171Pro
Asn163Asp  +  Ala164Asp  +  Thr167Ser
Ala164Asp  +  Gly165Glu  +  Asn166Gln
Gly162Ser  +  Ala164Glu  +  Gly165Asp
Gly165Asn  +  Asn170Glu  +  Tyr171Glu
Gly165Pro  +  Asn170Glu  +  Tyr171Glu
```

TABLE 17

Loop 4 - Quadruple Mutation Variants

```
Ala164Asn  +  Asn166Asp  +  Thr167Asn  +  Ala168Gln
Gly162Asn  +  Asn163Gln  +  Ala164Asn  +  Asn170Glu
Ala164Thr  +  Asn166Ser  +  Ala168Asn  +  Pro169Glu
Ala164Gln  +  Pro169Asn  +  Asn170Glu  +  Tyr171Thr
Asn163Ser  +  Ala164Thr  +  Thr167Asp  +  Tyr171Thr
Thr167Ser  +  Pro169Ser  +  Asn170Asp  +  Tyr171Ser
Gly162Asn  +  Thr167Gly  +  Pro169Gln  +  Tyr171Ser
Gly162Pro  +  Asn163Ser  +  Ala168Glu  +  Tyr171Val
Asn163Gln  +  Ala168Asp  +  Pro169Gln  +  Asn170Gln
Ala164Asn  +  Gly165Ser  +  Pro169Gln  +  Tyr171Ile
Gly162Glu  +  Thr167Gln  +  Ala168Ser  +  Tyr171Ser
Gly165Ser  +  Ala168Gly  +  Asn170Asp  +  Tyr171Leu
Ala164His  +  Gly165Asp  +  Asn166Gln  +  Tyr171Thr
Asn163Gln  +  Ala164Ser  +  Asn166Ser  +  Ala168Glu
Asn166Asp  +  Thr167Asn  +  Asn170Ser  +  Tyr171Thr
Gly162Asp  +  Gly165Gln  +  Asn170Ser  +  Tyr171Gln
Gly162Pro  +  Ala168Asn  +  Pro169Glu  +  Tyr171Gly
Asn166Gln  +  Thr167Gln  +  Ala168Thr  +  Asn170Asp
Asn163Gln  +  Thr167Asp  +  Ala168Ser  +  Tyr171Pro
```

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```
Ala164Pro + Thr167Glu + Pro169Ser + Tyr171Val
Gly165Asn + Asn166Gln + Pro169Gly + Asn170Asp
Asn166Ser + Thr167Pro + Ala168Asn + Asn170Glu
Ala164Gly + Ala168Asn + Pro169Asp + Tyr171Ala
Ala164Gln + Gly165Gln + Thr167Ser + Pro169Glu
Asn163Glu + Gly165Ser + Thr167Gln + Ala168Thr
Gly162Pro + Ala164Thr + Thr167Ser + Pro169Gln
Gly162Gln + Asn163Glu + Gly165Gln + Thr167Pro
Asn163Ser + Pro169Gly + Asn170Glu + Tyr171Ser
Asn163Gln + Gly165Ser + Asn170Asp + Tyr171Leu
Ala164His + Gly165Pro + Pro169Ser + Asn170Gln
Gly162Pro + Asn166Gln + Ala168Asn + Asn170Glu
Asn163Gln + Gly165Pro + Thr167Ser + Tyr171Gly
Asn163Gln + Gly165Asn + Asn166Ser + Tyr171Cys
Asn163Asp + Gly165Pro + Asn166Ser + Tyr171Gly
Ala164Pro + Thr167Pro + Pro169Glu + Asn170Ser
Asn163Asp + Ala164Glu + Pro169Gln + Tyr171His
Asn163Glu + Ala164Glu + Ala168Gly + Tyr171His
Thr167Asp + Ala168Glu + Pro169Gln + Asn170Ser
Ala164Asp + Gly165Asp + Ala168Asn + Pro169Gln
Gly162Ser + Ala164Gly + Asn170Asp + Tyr171Glu
Asn163Gln + Thr167Asn + Ala168Asp + Pro169Glu
Asn163Ser + Gly165Pro + Ala168Asp + Pro169Glu
Gly165Ser + Ala168Asp + Pro169Glu + Asn170Ser
Asn166Gln + Ala168Gln + Pro169Asp + Asn170Asp
Ala164Gly + Thr167Gly + Pro169Glu + Asn170Asp
Gly162Pro + Ala164Gly + Pro169Asp + Asn170Glu
Ala164Pro + Thr167Glu + Ala168Asp + Pro169Asp
Gly162Asp + Asn163Gln + Ala168His + Asn170Asp
Gly162Glu + Ala164Ser + Asn166Ser + Asn170Glu
Gly162Glu + Ala164Ser + Asn166Gln + Asn170Glu
Thr167Asp + Ala168His + Pro169Glu + Tyr171Pro
Asn163Glu + Ala164His + Gly165Asp + Asn166Gln
Asn163Asp + Ala164Gln + Gly165Asp + Asn166Ser
Asn163Glu + Ala164Asp + Gly165Gln + Ala168Asp
Gly162Glu + Ala164Glu + Asn166Ser + Pro169Asp
Ala164Glu + Asn166Asp + Asn170Ser + Tyr171Gly
Ala164Glu + Gly165Gln + Asn166Glu + Tyr171Cys
Ala164Asp + Asn166Glu + Thr167Asn + Pro169Asn
Thr167Asp + Pro169Glu + Asn170Glu + Tyr171Ser
Asn166Asp + Thr167Ser + Ala168Asp + Tyr171His
```

TABLE 18

Loop 5 - Single Mutation Variants

Ser191Asp
Ser191Glu
Ser192Asp
Ser192Glu
Phe193Ala
Phe193Asn
Phe193Asp

34

```
                    Phe193Cys
                    Phe193Gln
                    Phe193Glu
                    Phe193Gly.
                    Phe193His
                    Phe193Ile
                    Phe193Leu
                    Phe193Met
                    Phe193Pro
                    Phe193Ser
                    Phe193Thr
                    Phe193Tyr
                    Phe193Val
                    Ser194Asp
                    Ser194Glu
                    Thr195Asn
                    Thr195Asp
                    Thr195Gln
                    Thr195Glu
                    Thr195Gly
                    Thr195Pro
                    Thr195Ser
```

TABLE 19

Loop 5 - Double Mutation Variants

```
          Ser192Asp  +  Phe193Ser
          Ser192Asp  +  Phe193Met
          Ser191Glu  +  Phe193Asn
          Ser191Asp  +  Phe193Ile
          Ser191Glu  +  Phe193Gln
          Ser191Asp  +  Thr195Ser
          Phe193Gly  +  Thr195Asp
          Ser192Asp  +  Phe193Cys
          Ser191Glu  +  Thr195Pro
          Ser191Glu  +  Phe193Met
          Phe193Thr  +  Thr195Glu
          Ser192Asp  +  Thr195Gln
          Phe193Asp  +  Thr195Asn
          Ser191Asp  +  Phe193Gln
          Phe193Gly  +  Thr195Pro
          Ser192Asp  +  Thr195Ser
          Ser191Glu  +  Phe193Ile
          Phe193Gln  +  Thr195Asn
          Ser191Glu  +  Thr195Gly
          Ser191Glu  +  Phe193His
          Ser191Asp  +  Phe193Leu
          Ser192Glu  +  Phe193Pro
          Phe193His  +  Thr195Glu
          Ser192Asp  +  Thr195Gly
          Ser192Glu  +  Phe193Ala
          Ser192Glu  +  Phe193Val
```

Ser192Asp + Phe193Leu
Ser192Glu + Phe193Asn
Phe193Tyr + Thr195Ser
Phe193His + Thr195Asn
Ser192Glu + Thr195Ser
Phe193Ile + Thr195Glu
Phe193Val + Thr195Asn
Phe193Met + Thr195Gln
Ser191Asp + Thr195Pro
Ser192Glu + Phe193Tyr
Phe193Ser + Thr195Asn
Ser192Glu + Phe193Gln
Ser191Asp + Phe193Ser
Ser192Asp + Phe193Ile
Ser192Asp + Phe193His
Ser192Asp + Phe193Gly
Ser191Glu + Phe193Ser
Ser192Glu + Thr195Gln
Ser191Glu + Thr195Ser
Ser191Asp + Thr195Asn
Ser191Glu + Phe193Val
Phe193Tyr + Thr195Asp
Phe193His + Thr195Ser
Ser192Glu + Phe193Ile
Phe193Met + Thr195Gly
Ser191Glu + Phe193Tyr
Ser191Asp + Thr195Gln
Ser192Asp + Phe193Asn
Ser192Asp + Phe193Thr
Ser192Glu + Phe193Cys
Phe193Asp + Thr195Gln
Phe193Val + Thr195Gln
Ser192Glu + Phe193Thr
Phe193Leu + Thr195Glu

TABLE 20

Loop 5 - Triple Mutation Variants

Ser192Glu +	Phe193Thr +	Thr195Gly
Ser191Glu +	Phe193His +	Thr195Ser
Ser192Asp +	Phe193Leu +	Thr195Asn
Ser191Asp +	Phe193Ala +	Thr195Ser
Ser192Asp +	Phe193Val +	Thr195Gln
Ser191Asp +	Phe193Ser +	Thr195Pro
Ser191Asp +	Phe193Ile +	Thr195Gly
Ser192Glu +	Phe193His +	Thr195Gly
Ser192Glu +	Phe193Leu +	Thr195Asn
Ser192Asp +	Phe193Gly +	Thr195Gln
Ser192Asp +	Phe193Ile +	Thr195Pro
Ser191Glu +	Phe193Asn +	Thr195Ser
Ser192Asp +	Phe193Met +	Thr195Asn
Ser191Glu +	Phe193Gly +	Thr195Asn

36

```
Ser192Glu + Phe193His + Thr195Asn
Ser192Asp + Phe193Met + Thr195Pro
Ser191Glu + Phe193Leu + Thr195Asn
Ser191Glu + Phe193Met + Thr195Asn
Ser192Glu + Phe193Ala + Thr195Gln
Ser191Glu + Phe193Tyr + Thr195Gly
Ser192Asp + Phe193Ala + Thr195Gly
Ser191Glu + Phe193Met + Thr195Gly
Ser192Glu + Phe193Thr + Thr195Gln
Ser191Asp + Phe193Asn + Thr195Gly
Ser192Glu + Phe193Asn + Thr195Ser
Ser192Asp + Phe193Ser + Thr195Gly
Ser192Glu + Phe193Leu + Thr195Pro
Ser191Asp + Phe193Cys + Thr195Gly
Ser191Asp + Ser192Asp + Phe193Tyr
Ser191Asp + Ser192Asp + Phe193Gly
Ser191Asp + Ser192Glu + Phe193Met
Ser191Asp + Ser192Glu + Thr195Gln
Ser191Glu + Ser192Asp + Phe193Val
Ser191Glu + Ser192Glu + Phe193Thr
Ser191Glu + Ser192Asp + Phe193Thr
Ser191Asp + Ser192Asp + Thr195Gly
Ser191Glu + Ser192Glu + Phe193Asn
Ser191Asp + Ser192Glu + Phe193Ala
Ser191Asp + Ser192Asp + Phe193Asn
Ser191Asp + Ser192Asp + Phe193Pro
Ser191Asp + Ser192Glu + Phe193Thr
Ser191Asp + Ser192Asp + Thr195Asn
Ser191Asp + Ser192Asp + Thr195Gln
Ser191Glu + Ser192Asp + Phe193Asn
Ser191Glu + Ser192Glu + Phe193Gln
Ser191Glu + Ser192Asp + Phe193Leu
Ser191Asp + Ser192Asp + Thr195Ser
Ser191Asp + Ser192Glu + Phe193Ser
Ser191Glu + Ser192Asp + Phe193Tyr
Ser191Asp + Ser192Asp + Phe193Thr
Ser191Asp + Ser192Glu + Phe193Pro
Ser191Glu + Ser192Asp + Phe193Ile
Ser191Asp + Ser192Glu + Phe193Tyr
Ser191Glu + Ser192Glu + Phe193Tyr
Ser191Asp + Ser192Asp + Phe193Ser
Ser191Glu + Ser192Glu + Thr195Pro
Ser191Asp + Ser192Glu + Thr195Ser
Ser191Glu + Ser192Asp + Phe193Met
Ser191Asp + Ser192Glu + Thr195Pro
Ser191Glu + Ser192Asp + Phe193His
```

TABLE 21

Loop 5 - Quadruple Mutation Variants

```
Ser191Asp + Ser192Glu + Phe193Gly + Thr195Gln
Ser191Glu + Ser192Asp + Phe193His + Thr195Gly
```

37

```
Ser191Glu + Ser192Glu + Phe193Cys + Thr195Pro
Ser191Asp + Ser192Glu + Phe193Cys + Thr195Gln
Ser191Asp + Ser192Glu + Phe193Thr + Thr195Pro
Ser191Glu + Ser192Glu + Phe193Val + Thr195Asn
Ser191Asp + Ser192Glu + Phe193Val + Thr195Asn
Ser191Glu + Ser192Glu + Phe193Leu + Thr195Ser
Ser191Glu + Ser192Asp + Phe193Cys + Thr195Gln
Ser191Glu + Ser192Asp + Phe193Pro + Thr195Pro
Ser191Glu + Ser192Asp + Phe193Ser + Thr195Gly
Ser191Glu + Ser192Asp + Phe193Pro + Thr195Asn
Ser191Glu + Ser192Glu + Phe193Ser + Thr195Pro
Ser191Glu + Ser192Asp + Phe193Ala + Thr195Gly
Ser191Asp + Ser192Glu + Phe193His + Thr195Asn
Ser191Asp + Ser192Glu + Phe193Asn + Thr195Asn
Ser191Glu + Ser192Glu + Phe193Gln + Thr195Ser
Ser191Glu + Ser192Glu + Phe193Thr + Thr195Gln
Ser191Glu + Ser192Glu + Phe193Asn + Thr195Gln
Ser191Glu + Ser192Glu + Phe193Tyr + Thr195Ser
Ser191Asp + Ser192Glu + Phe193Ile + Thr195Pro
Ser191Glu + Ser192Asp + Phe193Val + Thr195Gln
Ser191Glu + Ser192Asp + Phe193Val + Thr195Asn
Ser191Asp + Ser192Glu + Phe193Tyr + Thr195Ser
Ser191Asp + Ser192Glu + Phe193Ala + Thr195Ser
Ser191Glu + Ser192Glu + Phe193His + Thr195Asn
Ser191Asp + Ser192Asp + Phe193Pro + Thr195Asn
Ser191Asp + Ser192Asp + Phe193Gln + Thr195Ser
Ser191Asp + Ser192Asp + Phe193Leu + Thr195Asn
Ser191Asp + Ser192Glu + Phe193Val + Thr195Gln
Ser191Glu + Ser192Glu + Phe193Gly + Thr195Gly
Ser191Glu + Ser192Glu + Phe193Thr + Thr195Pro
Ser191Glu + Ser192Glu + Phe193Thr + Thr195Gly
Ser191Glu + Ser192Asp + Phe193Ile + Thr195Ser
Ser191Glu + Ser192Glu + Phe193Ile + Thr195Gly
Ser191Asp + Ser192Asp + Phe193Pro + Thr195Pro
Ser191Asp + Ser192Asp + Phe193Ile + Thr195Gly
Ser191Glu + Ser192Asp + Phe193Thr + Thr195Gly
Ser191Asp + Ser192Glu + Phe193Ser + Thr195Gly
Ser191Glu + Ser192Asp + Phe193Tyr + Thr195Ser
Ser191Asp + Ser192Asp + Phe193Thr + Thr195Gln
Ser191Asp + Ser192Glu + Phe193Ser + Thr195Gln
Ser191Glu + Ser192Glu + Phe193His + Thr195Ser
Ser191Glu + Ser192Asp + Phe193Leu + Thr195Asn
Ser191Asp + Ser192Glu + Phe193Leu + Thr195Pro
Ser191Asp + Ser192Asp + Phe193Ile + Thr195Pro
Ser191Asp + Ser192Asp + Phe193Tyr + Thr195Ser
Ser191Asp + Ser192Glu + Phe193Ile + Thr195Gln
Ser191Asp + Ser192Glu + Phe193Pro + Thr195Ser
Ser191Asp + Ser192Glu + Phe193Leu + Thr195Gln
Ser191Glu + Ser192Asp + Phe193Asn + Thr195Ser
Ser191Glu + Ser192Glu + Phe193Ser + Thr195Asn
Ser191Asp + Ser192Asp + Phe193Glu + Thr195Gln
Ser191Asp + Ser192Glu + Phe193Glu + Thr195Pro
Ser191Glu + Ser192Asp + Phe193Glu + Thr195Gln
```

38

```
Ser191Glu + Ser192Glu + Phe193Glu + Thr195Gly
Ser191Glu + Ser192Glu + Phe193Asp + Thr195Gly
Ser191Asp + Ser192Asp + Phe193Glu + Thr195Ser
Ser191Glu + Ser192Asp + Phe193Asp + Thr195Pro
Ser191Glu - Ser192Glu + Phe193Asp + Thr195Gln
```

TABLE 22

Loop 6 - Single Mutation Variants

Ala204Asn
Ala204Asp
Ala204Gln
Ala204Glu
Ala204Gly
Ala204His
Ala204Pro
Ala204Ser
Ala204Thr
Pro205Asn
Pro205Asp
Pro205Gln
Pro205Glu
Pro205Gly
Pro205Ser
Gly206Asn
Gly206Asp
Gly206Gln
Gly206Glu
Gly206Pro
Gly206Ser
Ser207Asp
Ser207Glu
Trp208Ala
Trp208Asn
Trp208Asp
Trp208Cys
Trp208Gln
Trp208Glu
Trp208Gly
Trp208His
Trp208Ile
Trp208Leu
Trp208Met
Trp208Phe
Trp208Pro
Trp208Ser
Trp208Thr
Trp208Tyr
Trp208Val
Ile209Ala
Ile209Asn
Ile209Asp

```
Ile209Cys
Ile209Gln
Ile209Glu
Ile209Gly
Ile209His
Ile209Leu
Ile209Met
Ile209Pro
Ile209Ser
Ile209Thr
Ile209Val
Tyr210Ala
Tyr210Asn
Tyr210Asp
Tyr210Cys
Tyr210Gln
Tyr210Glu
Tyr210Gly
Tyr210His
Tyr210Ile
Tyr210Leu
Tyr210Met
Tyr210Pro
Tyr210Ser
Tyr210Thr
Tyr210Val
Ser211Asp
Ser211Glu
Thr212Asn
Thr212Asp
Thr212Gln
Thr212Glu
Thr212Gly
Thr212Pro
Thr212Ser
Tyr213Ala
Tyr213Asn
Tyr213Asp
Tyr213Cys
Tyr213Gln
Tyr213Glu
Tyr213Gly
Tyr213His
Tyr213Ile
Tyr213Leu
Tyr213Met
Tyr213Pro
Tyr213Ser
Tyr213Thr
Tyr213Val
Pro214Asn
Pro214Asp
Pro214Gln
```

40

```
Pro214Glu
Pro214Gly
Pro214Ser
Thr215Asn
Thr215Asp
Thr215Gln
Thr215Glu
Thr215Gly
Thr215Pro
Thr215Ser
Ser216Asp
Ser216Glu
Thr217Asn
Thr217Asp
Thr217Gln
Thr217Glu
Thr217Gly
Thr217Pro
Thr217Ser
Tyr218Ala
Tyr218Asn
Tyr218Asp
Tyr218Cys
Tyr218Gln
Tyr218Glu
Tyr218Gly
Tyr218His
Tyr218Ile
Tyr218Leu
Tyr218Met
Tyr218Pro
Tyr218Ser
Tyr218Thr
Tyr218Val
Ala219Asn
Ala219Asp
Ala219Gln
Ala219Glu
Ala219Gly
Ala219His
Ala219Pro
Ala219Ser
Ala219Thr
Ser220Asp
Ser220Glu
Leu221Ala
Leu221Asn
Leu221Asp
Leu221Cys
Leu221Gln
Leu221Glu
Leu221Gly
Leu221His
```

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Leu221Ile
Leu221Met
Leu221Pro
Leu221Ser
Leu221Thr
Leu221Val
Ser222Asp
Ser222Glu
Gly223Asn
Gly223Asp
Gly223Gln
Gly223Glu
Gly223Pro
Gly223Ser
Thr224Asn
Thr224Asp
Thr224Gln
Thr224Glu
Thr224Gly
Thr224Pro
Thr224Ser

TABLE 23

Loop 6 - Double Mutation Variants

Ser207Glu + Thr215Gln
Pro205Gly + Ala219Gly
Thr217Asn + Thr224Gly
Gly206Gln + Pro214Asp
Ala204Asn + Thr212Ser
Pro205Gly + Ser220Asp
Tyr213Gln + Tyr218Glu
Pro205Gln + Leu221Glu
Thr212Gln + Ser220Asp
Tyr210Gly + Thr224Gly
Tyr213Gly + Ser216Glu
Ser207Glu + Gly223Asn
Tyr210Ser + Tyr218Leu
Ser216Glu + Thr217Ser
Ser216Glu + Thr224Asn
Pro205Asn + Ala219Asn
Thr217Gly + Leu221Thr
Thr215Glu + Ala219Gln
Thr217Asn + Tyr218Glu
Ala204Asn + Gly206Pro
Thr212Ser + Thr224Gln
Thr217Gly + Ser222Glu
Trp208Glu + Ile209Asn
Pro205Ser + Ala219Gly
Tyr213Ser + Ala219Ser
Ala204Ser + Gly206Ser
Ala204Gly + Thr215Glu

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```
Thr212Gln + Ala219Glu
Ala204Gly + Trp208Gln
Ala204Thr + Ser216Asp
Thr212Gln + Tyr218Gln
Ser207Glu + Leu221Met
Thr215Pro + Gly223Gln
Ala204Gly + Ser216Asp
Tyr210Asp + Ala219His
Ile209Gln + Tyr218Ala
Ile209Ala + Ala219Pro
Ala204Asn + Ser222Glu
Tyr210Asp + Tyr213Ala
Tyr218Leu + Thr224Glu
Tyr218Asn + Ser220Glu
Trp208Met + Ala219Pro
Pro205Gly + Ser222Asp
Leu221Val + Thr224Gln
Trp208Ile + Pro214Ser
Trp208Asp + Thr212Pro
Ala204Thr + Gly223Gln
Thr217Gln + Tyr218Ile
Tyr213His + Ser216Asp
Thr212Ser + Thr215Asp
Thr217Asn + Leu221Asn
Pro214Asp + Thr217Pro
Ser207Glu + Ala219His
Thr217Asp + Tyr218His
Trp208Ala + Thr217Ser
Pro205Gln + Ser220Asp
Ile209Gly + Ser216Glu
Tyr218Ser + Ser220Glu
Thr215Asn + Ser216Glu
Thr215Gly + Tyr218Gln
Thr217Ser + Leu221Ala
Ala219Pro + Leu221His
Pro205Gln + Leu221Met
Tyr213Met + Leu221Ile
Trp208Asn + Thr212Ser
Ser207Glu + Thr224Gly
Pro205Ser + Leu221Cys
Ala204Gly + Tyr210Val
Trp208Phe + Ser222Asp
Ile209Thr + Ser222Glu
Leu221Ile + Ser222Glu
Tyr213Pro + Thr217Glu
Ile209Thr + Ser216Glu
Tyr210Cys + Ser222Asp
Leu221Glu + Thr224Ser
Tyr213Ala + Thr217Pro
Trp208Cys + Thr215Glu
Ala204Ser + Thr217Ser
Ile209His + Gly223Ser
Ala204Asn + Trp208Tyr
```

```
Pro205Gln  +  Ser207Glu
Ala204Asn  +  Trp208Met
Tyr210Pro  +  Leu221Asp
Tyr210Gln  +  Thr215Ser
Ala204Pro  +  Ser216Glu
Ala204Gly  +  Ser222Asp
Ser216Asp  +  Leu221Met
Tyr210Ile  +  Ala219Asn
Pro214Glu  +  Thr224Ser
Pro205Gln  +  Thr215Ser
Ala204Gly  +  Pro205Asn
Tyr210Asp  +  Tyr218Val
Trp208Ser  +  Thr215Gly
Ser222Glu  +  Thr224Ser
Trp208Gln  +  Ser216Asp
Ser207Asp  +  Gly223Ser
Pro205Gly  +  Tyr210Cys
Thr212Gly  +  Thr224Asp
Ser220Glu  +  Gly223Pro
Gly206Pro  +  Tyr210Met
Tyr210Ser  +  Ser222Asp
Ser207Glu  +  Pro214Gln
Pro205Gly  +  Gly223Asn
Tyr213Ser  +  Pro214Asp
Pro214Gln  +  Ser220Asp
Ala204Thr  +  Ser222Glu
Ala219Asn  +  Leu221Asp
Gly206Ser  +  Thr215Pro
Thr212Asn  +  Tyr218Val
Thr217Pro  +  Gly223Asp
Gly206Pro  +  Tyr218Asn
Gly206Gln  +  Thr217Asp
Tyr210Ala  +  Ser220Asp
Pro214Asp  +  Tyr218Val
Leu221His  +  Gly223Asp
Pro214Gln  +  Gly223Asp
Ile209Pro  +  Ser216Asp
Ile209Thr  +  Gly223Asp
Pro214Asn  +  Ala219Asp
Tyr218Cys  +  Ser220Glu
Ser207Glu  +  Tyr218Asn
Tyr218Gly  +  Leu221Gln
Ile209Thr  +  Tyr218Val
Pro214Glu  +  Gly223Ser
Tyr213Met  +  Ala219Thr
Ser222Glu  +  Thr224Asn
Trp208Asp  +  Ile209Met
Thr212Gln  +  Pro214Gln
Ser207Asp  +  Tyr213Leu
Thr212Gln  +  Gly223Pro
Tyr210Leu  +  Ser216Glu
Ser216Asp  +  Ala219Thr
Tyr213Gln  +  Thr217Asp
```

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```
Gly206Pro + Ser222Glu
Ala204Thr + Ser220Asp
Ala219Thr + Thr224Pro
Trp208Ile + Ala219Glu
Ile209Gly + Thr215Ser
Gly206Gln + Tyr210Glu
Pro214Gln + Ser220Glu
Ser207Glu + Thr217Asn
Ala204Pro + Gly223Asp
Ile209Cys + Ser222Asp
Pro214Gly + Gly223Pro
Ile209Met + Thr224Pro
Pro214Glu + Thr217Ser
Pro205Ser + Ser207Asp
Tyr210Thr + Thr212Gln
Gly206Gln + Tyr218Pro
Tyr218Val + Ser222Glu
Leu221Asn + Gly223Glu
Pro205Gln + Tyr218Val
Pro205Gln + Thr217Asn
Trp208His + Ile209Ala
Pro205Ser + Gly223Gln
Pro214Ser + Thr217Gly
Tyr218His + Ser222Asp
Thr212Gln + Gly223Ser
Thr215Glu + Ala219Thr
Tyr213Gly + Tyr218Asp
Thr212Gln + Tyr213Gln
Tyr210Ile + Thr215Asp
Ala204Asn + Gly223Pro
Ser207Glu + Ile209Asn
Thr215Gln + Ala219Asp
Ser220Asp + Gly223Pro
Pro205Ser + Tyr213Ala
Trp208Gly + Ser222Glu
Thr215Glu + Tyr218Ala
Ala204Gln + Ala219Gln
Pro205Asn + Tyr213Thr
Tyr218Thr + Thr224Gln
Ser222Asp + Thr224Ser
Ala204Pro + Tyr213Cys
Gly206Gln + Leu221Gly
Ser216Glu + Thr224Ser
Ser207Asp + Ala219Asn
Trp208Leu + Ile209His
Pro205Ser + Tyr213Val
Thr212Gly + Thr215Asp
```

TABLE 24

Loop 6 - Triple Mutation Variants

```
Gly206Ser + Thr212Ser + Ala219Glu
```

```
Ala204Asn + Thr212Ser + Ser216Glu
Pro205Gln + Leu221Glu + Gly223Asn
Trp208Thr + Ile209Cys + Thr224Asp
Ile209Met + Thr212Gln + Ser220Asp
Tyr210Gly + Tyr213Gly + Thr224Gly
Ser207Glu + Tyr210Ser + Gly223Asn
Tyr213Gly + Ser216Asp + Thr217Ser
Pro205Asn + Ser216Glu + Thr224Asn
Thr215Glu + Ala219Gln + Leu221Thr
Ala204Asn + Thr217Asn + Tyr218Glu
Thr212Ser + Thr217Gly + Thr224Gln
Ala204Ser + Tyr218His + Ala219Gln
Pro205Ser + Tyr213Ser + Leu221Asn
Ala204Ser + Gly206Ser + Ala219Ser
Ala204Gly + Trp208Gln + Ser216Asp
Ser207Glu + Tyr218Gln + Leu221Met
Ala204Gly + Thr215Pro + Gly223Gln
Ile209Gln + Tyr218Ala + Ala219Pro
Ala204Asn + Trp208Met + Ser222Glu
Trp208Met + Ala219Pro + Ser222Asp
Pro205Gly + Leu221Val + Thr224Gln
Ala204Thr + Thr212Pro + Gly223Gln
Gly206Gln + Ile209Gln + Thr212Gln
Gly206Pro + Ser220Glu + Leu221Ser
Gly206Gln + Tyr210Gly + Ser222Asp
Ala204Gly + Tyr213Thr + Pro214Gln
Ala204His + Thr212Gln + Thr224Gln
Gly206Gln + Pro214Asp + Tyr218Ser
Thr212Asn + Ser216Asp + Thr217Ser
Ser207Asp + Tyr213Ile + Thr224Pro
Pro205Gln + Tyr213Met + Leu221His
Trp208Asn + Thr212Ser + Gly223Asp
Ser207Glu + Leu221Gly + Thr224Gly
Pro205Ser + Tyr210Val + Leu221Cys
Pro205Gly + Ile209His + Tyr213Thr
Ile209Pro + Tyr210Asn + Ser220Asp
Pro205Gly + Thr212Pro + Thr224Gly
Trp208Cys + Thr217Asp + Ala219Asn
Pro205Gly + Leu221Cys + Gly223Pro
Ala204His + Ser216Glu + Leu221Ser
Thr217Asp + Gly223Ser + Thr224Gly
Pro205Gln + Trp208Gln + Ile209Ala
Thr212Gly + Thr217Gly + Leu221His
Gly206Asn + Thr212Gly + Tyr213Gly
Gly206Asn + Ser207Glu + Thr212Ser
Thr212Gln + Leu221Glu + Gly223Ser
Tyr210His + Thr215Asp + Thr224Pro
Pro214Gln + Thr215Pro + Ser216Glu
Pro205Gly + Tyr213Val + Gly223Asp
Trp208Phe + Thr215Asn + Thr224Gly
Tyr213Met + Ala219Pro + Ser220Glu
Trp208Phe + Ile209Ser + Tyr213Gln
Gly206Ser + Ser216Glu + Tyr218Pro
```

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```
Ala204Gln + Tyr210His + Thr224Glu
Pro205Ser + Thr212Pro + Ala219Ser
Pro205Gln + Tyr210Met + Thr217Pro
Ala204Gly + Ile209His + Ser216Glu
Thr212Asn + Thr217Ser + Thr224Asn
Gly206Pro + Tyr210Gly + Gly223Gln
Pro205Gln + Thr217Asp + Ala219Thr
Pro205Ser + Ile209Met + Ser216Asp
Thr215Asn + Ser216Glu + Thr224Ser
Pro205Gln + Leu221Thr + Ser222Glu
Tyr210Cys + Ala219Asn + Leu221Thr
Ala204Ser + Gly206Ser + Ala219Gln
Ile209Pro + Ser216Asp + Leu221Cys
Trp208Thr + Tyr210Val + Thr212Ser
Thr212Gly + Pro214Asp + Gly223Gln
Pro214Gln + Thr215Ser + Thr224Glu
Thr212Ser + Pro214Gln + Leu221His
Trp208Gly + Ser220Glu + Gly223Ser
Pro205Ser + Ser220Asp + Leu221Thr
Gly206Asn + Thr215Pro + Leu221Ala
Tyr213Leu + Leu221His + Gly223Asp
Ser207Asp + Thr212Gly + Tyr218Cys
Gly206Gln + Ile209Val + Ala219Thr
Trp208Ile + Tyr218Ile + Gly223Asn
Ala204His + Ile209Gly + Ser216Asp
Trp208Leu + Thr212Gly + Ala219Ser
Thr212Gln + Pro214Glu + Ala219Thr
Pro205Gln + Ser207Asp + Thr212Pro
Pro205Ser + Tyr210Pro + Tyr218Ile
Leu221Ala + Gly223Asn + Thr224Glu
Ala204Thr + Gly206Gln + Ser207Asp
Ser216Glu + Ala219Gln + Gly223Gln
Pro205Gly + Trp208Leu + Leu221Gln
Thr217Gln + Gly223Gln + Thr224Glu
Thr212Gly + Thr217Ser + Tyr218Ala
Gly206Asn + Gly223Asp + Thr224Asn
Ala204Gly + Tyr210Cys + Tyr213Thr
Gly206Ser + Thr212Asn + Thr215Gly
Ala204Asn + Thr212Asn + Ser216Glu
Gly206Gln + Tyr218Cys + Gly223Glu
Ala204Ser + Pro205Ser + Gly206Asn
Ile209Gly + Leu221Val + Thr224Pro
Gly206Asn + Trp208His + Thr217Gly
Gly206Ser + Trp208Leu + Ser222Asp
Pro205Gly + Thr212Gln + Ser222Asp
Ala204Thr + Tyr213Gln + Thr224Pro
Thr212Ser + Thr217Ser + Gly223Asp
Pro205Ser + Tyr213Ile + Ser222Glu
Ile209Pro + Tyr210Asp + Ala219Pro
Gly206Gln + Thr215Pro + Ser220Glu
Trp208Cys + Tyr210Leu + Thr215Gly
Trp208Tyr + Thr212Gln + Ser222Glu
Gly206Asn + Ser222Asp + Gly223Asn
```

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```
Pro205Gly + Gly206Asn + Leu221Cys
Pro205Gln + Tyr213Ile + Ser216Glu
Ala204His + Tyr210His + Ser220Asp
Ala204Thr + Thr212Asn + Ala219Asp
Ser207Glu + Tyr210Ala + Thr212Gly
Tyr210His + Thr217Gly + Leu221Met
Ala204Thr + Ile209Met + Ser222Glu
Trp208Pro + Ala219Pro + Ser222Asp
Ala204His + Ile209Ser + Ser220Glu
Pro214Ser + Leu221Glu + Gly223Gln
Gly206Pro + Leu221Met + Gly223Asp
Ala204His + Tyr213Thr + Ser222Asp
Tyr213Ala + Leu221Met + Ser222Asp
Trp208Leu + Thr212Ser + Pro214Asp
Tyr213Ile + Ser216Glu + Thr217Pro
Pro205Gln + Pro214Gln + Thr224Gly
Tyr213Cys + Thr217Gly + Ser220Glu
Gly206Asn + Thr212Gly + Tyr218Pro
Gly206Pro + Ser216Glu + Gly223Pro
Thr212Gln + Thr215Gly + Ser222Glu
Ala204Pro + Thr217Asp + Thr224Ser
Pro205Ser + Thr212Pro + Ala219Asp
Ile209Cys + Pro214Ser + Thr215Asp
Ile209Cys + Ser220Asp + Gly223Gln
Thr217Gly + Ser220Asp + Leu221Gln
Ala204His + Pro205Asn + Trp208Glu
Ala204Gln + Thr212Ser + Thr215Asp
Pro214Asn + Ser220Glu + Leu221Gly
Ile209Gly + Thr215Asn + Ala219Pro
Ser207Glu + Thr217Gln + Thr224Ser
Ala204Gly + Thr215Asn + Gly223Pro
Ile209Ser + Tyr213Ile + Leu221Met
Tyr210Thr + Pro214Ser + Ser222Asp
Tyr210Leu + Thr212Ser + Leu221Asn
Gly206Asn + Pro214Asp + Tyr218Met
Gly206Asn + Pro214Gln + Ala219Asn
Ser207Glu + Pro214Ser + Ala219His
Ala204Asn + Trp208Ala + Ile209Gln
Thr217Glu + Tyr218Leu + Gly223Asn
Trp208Pro + Pro214Gly + Leu221Ala
Gly206Pro + Thr215Ser + Leu221Cys
Ala204Ser + Ile209Val + Thr224Asp
Trp208Ile + Tyr210Pro + Thr224Asp
Thr212Gln + Thr215Asn + Thr217Asp
Tyr210Ser + Tyr213Cys + Thr217Asn
Thr212Gln + Ser222Asp + Gly223Gln
Tyr210Asn + Thr217Glu + Tyr218Met
Pro214Asn + Ala219Gln + Ser220Glu
Pro205Ser + Trp208Met + Leu221Asp
Ala204Asn + Tyr210Cys + Tyr213Asn
Tyr218Leu + Leu221His + Ser222Glu
Ala204His + Tyr218Ser + Ser222Asp
Gly206Pro + Tyr213Asn + Pro214Asn
```

```
Gly206Pro + Thr215Gln + Tyr218Ser
Ala204Pro + Ile209His + Leu221Thr
Gly206Gln + Thr212Gly + Gly223Glu
Thr215Gly + Tyr218Ala + Thr224Pro
Pro205Gln + Tyr213Gly + Gly223Glu
Ala204Gly + Tyr213Asn + Leu221Thr
Trp208Gly + Ile209His + Thr215Ser
Ala204His + Ser207Glu + Tyr213Cys
Trp208His + Tyr210His + Ser222Asp
Pro205Gly + Tyr210Asp + Leu221Gln
Pro205Asn + Tyr210Ala + Pro214Ser
Thr212Pro + Thr215Gln + Thr224Asp
Ala204Thr + Tyr213Val + Pro214Glu
Ala204Gly + Tyr210Pro + Pro214Glu
Pro205Gly + Trp208His + Tyr218Gly
Gly206Ser + Ala219Gln + Gly223Ser
Tyr213Thr + Tyr218Cys + Ser220Glu
Thr215Pro + Ser216Glu + Leu221Asn
Pro205Gly + Thr212Asn + Thr224Asp
Pro205Gly + Ser207Asp + Thr212Ser
```

TABLE 25

Loop 6 - Quadruple Mutation Variants			
Gly206Asn +	Thr215Gln +	Tyr218Ile +	Leu221Ile
Tyr210Gly +	Thr212Gln +	Tyr213Gly +	Thr224Gly
Pro205Gly +	Gly206Pro +	Trp208Asn +	Thr224Ser
Ile209Thr +	Pro214Asp +	Thr217Asn +	Tyr218Cys
Ala204Ser +	Tyr218His +	Ala219Gln +	Leu221Asn
Ala204Ser +	Pro205Ser +	Tyr213Ser +	Ala219Ser
Ala204Gly +	Thr212Gln +	Ser216Asp +	Gly223Ser
Ser207Glu +	Thr215Pro +	Tyr218Gln +	Leu221Met
Ala204Gly +	Ser216Asp +	Ala219His +	Gly223Gln
Ile209Gln +	Tyr210Asp +	Tyr218Ala +	Ala219Pro
Trp208Met +	Tyr218Leu +	Ala219Pro +	Ser222Glu
Pro205Gly +	Leu221Val +	Ser222Asp +	Thr224Gln
Ala204Thr +	Thr212Pro +	Ser222Glu +	Gly223Gln
Gly206Gln +	Ile209Gln +	Thr212Gln +	Leu221His
Ala204Ser +	Gly206Pro +	Ser220Asp +	Leu221Ser
Ala204Gly +	Thr212Gln +	Tyr213Thr +	Thr224Gln
Tyr210Ala +	Pro214Ser +	Tyr218Val +	Ser222Asp
Ala204Gln +	Pro205Ser +	Gly206Ser +	Ser222Glu
Trp208Cys +	Pro214Gln +	Ala219Thr +	Thr224Asp
Ala204Gly +	Gly206Ser +	Tyr210Asp +	Leu221Gly
Ala204Gln +	Gly206Asn +	Tyr210Gln +	Ala219Asp
Pro205Ser +	Gly206Pro +	Ile209Val +	Tyr213Pro
Gly206Pro +	Pro214Glu +	Thr215Pro +	Leu221Met
Ser207Asp +	Trp208Met +	Tyr218Val +	Leu221Pro
Tyr210Gly +	Thr212Pro +	Pro214Gly +	Ser222Asp
Gly206Pro +	Thr212Gln +	Thr217Ser +	Ser222Asp
Ala204Pro +	Trp208Met +	Ile209Val +	Ser216Glu
Trp208Ser +	Tyr218His +	Ala219His +	Gly223Pro

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```
Ala204Gln + Trp208Ala + Tyr210Asn + Ser222Glu
Gly206Gln + Ser220Asp + Leu221Ala + Thr224Ser
Ile209Gln + Thr212Ser + Ala219Gln + Ser220Glu
Pro205Asn + Thr212Gly + Tyr218Ala + Ser222Glu
Pro205Gln + Trp208Cys + Tyr213Cys + Pro214Asp
Pro205Gly + Thr215Gln + Thr217Pro + Gly223Ser
Ala204His + Gly206Pro + Ser216Glu + Leu221Ser
Ser207Asp + Leu221Gly + Gly223Gln + Thr224Asn
Tyr210His + Thr212Ser + Leu221Glu + Gly223Ser
Pro205Gly + Thr215Asn + Gly223Asp + Thr224Gly
Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu
Gly206Ser + Trp208Phe + Ile209Ser + Tyr213Gln
Ala204Gln + Pro205Ser + Thr212Pro + Ala219Ser
Ala204Gly + Tyr210Met + Ser216Glu + Thr217Pro
Gly206Pro + Thr212Asn + Thr217Gln + Thr224Asn
Pro205Ser + Ile209Met + Thr215Asn + Thr224Ser
Tyr210Thr + Thr212Pro + Tyr218Asn + Ser220Glu
Trp208Thr + Tyr210Val + Thr212Ser + Leu221Cys
Thr212Gly + Pro214Asp + Thr215Ser + Gly223Gln
Ser207Glu + Thr212Ser + Pro214Gln + Leu221Pro
Pro205Asn + Gly206Gln + Ser207Asp + Leu221Thr
Pro205Ser + Thr215Pro + Ser220Asp + Leu221Cys
Gly206Asn + Tyr213Leu + Leu221Ala + Gly223Asp
Ser207Asp + Thr212Gly + Pro214Asn + Thr224Asn
Ala204Pro + Ser207Glu + Ile209Val + Ala219Thr
Gly206Gln + Trp208Ile + Tyr218Ile + Gly223Asn
Ala204His + Trp208Leu + Ile209Gly + Ser216Asp
Ala204Gln + Thr212Gly + Pro214Glu + Ala219Ser
Ala204Asn + Pro205Gln + Ser207Asp + Thr212Pro
Pro205Gly + Trp208Leu + Ser216Glu + Ala219Gln
Trp208Tyr + Thr217Gln + Leu221Gln + Thr224Glu
Thr212Gly + Thr217Ser + Tyr218Ala + Gly223Asp
Ala204Gly + Gly206Asn + Thr215Asn + Gly223Asp
Gly206Ser + Tyr210Cys + Tyr213Thr + Thr215Gly
Ala204Gln + Pro205Ser + Gly206Asn + Ala219Asp
Ala204Ser + Ile209Gly + Leu221Val + Thr224Pro
Gly206Asn + Ser207Glu + Trp208His + Thr217Gly
Gly206Ser + Trp208Pro + Ala219Thr + Ser222Asp
Ala204Thr + Tyr213Gln + Thr217Ser + Thr224Pro
Pro205Ser + Thr212Ser + Tyr213Ile + Gly223Asp
Gly206Gln + Ile209Asn + Thr215Pro + Ser220Glu
Trp208Cys + Thr212Gln + Thr215Gly + Ser222Glu
Gly206Asn + Trp208Tyr + Ser222Glu + Gly223Asn
Pro205Gly + Gly206Asn + Leu221Cys + Ser222Asp
Pro205Gln + Tyr210His + Tyr213Ile + Ser220Asp
Ala204His + Trp208Asn + Thr212Asn + Ala219Asp
Ala204His + Gly206Pro + Leu221Met + Gly223Asp
Ala204Ser + Tyr213Thr + Leu221Met + Ser222Asp
Tyr213Ile + Pro214Gln + Ser216Glu + Thr217Pro
Pro205Gln + Tyr213Cys + Ser220Glu + Thr224Gly
Gly206Asn + Tyr213Asn + Tyr218Pro + Ser220Glu
Pro205Ser + Gly206Pro + Tyr218Asp + Gly223Pro
Ala204Pro + Pro205Ser + Thr217Asp + Thr224Ser
```

```
Pro205Asn - Trp208Ile + Thr212Gln + Thr217Gly
Ile209Cys + Pro214Ser + Ser220Asp + Gly223Gln
Ala204His - Pro205Asn + Trp208Glu + Tyr210His
Ile209Gly + Thr215Asn + Thr217Asn + Ala219Pro
Pro205Gly - Pro214Asn + Tyr218Ile + Ser222Asp
Ala204Ser + Pro205Ser + Thr212Asn + Tyr213Ile
Pro205Gln + Ser207Glu + Tyr210Pro + Thr215Ser
Pro205Gln + Ser207Glu + Thr212Gln + Tyr213Ser
Ala204Ser - Ile209Asn + Tyr213Cys + Ser216Asp
Trp208Ala + Ile209Gln + Thr217Glu + Gly223Asn
Trp208Pro + Pro214Gly + Tyr218Leu + Leu221Ala
Gly206Pro + Thr215Ser + Ser216Glu + Leu221Cys
Ala204Ser + Trp208Ile + Ile209Val + Thr224Asp
Tyr210Pro + Thr212Gln + Thr215Asn + Thr217Asp
Tyr210Ser + Tyr213Cys + Pro214Asn + Thr217Asn
Thr212Gln + Tyr218Met + Ser222Asp + Gly223Gln
Pro205Ser + Trp208Met + Ala219Gln + Leu221Asp
Ala204Asn + Tyr210Cys + Tyr213Asn + Tyr218Leu
Ala204His + Pro205Gly + Tyr218Ser + Ser222Asp
Gly206Pro + Tyr213Asn + Pro214Asn + Tyr218Ser
Ala204Pro + Ile209His + Thr215Gln + Leu221Thr
Ala204Asn + Gly206Gln + Thr212Gly + Gly223Glu
Thr215Gly + Tyr218Ala + Gly223Glu + Thr224Pro
Pro205Gln + Tyr213Gly + Ser220Asp + Leu221Thr
Pro205Gly + Trp208His + Tyr210His + Leu221Gln
Tyr210Leu + Thr212Pro + Tyr218Gln + Ala219Glu
Gly206Gln + Thr212Gln + Tyr218His + Gly223Asp
Trp208Ile + Tyr213His + Thr215Ser + Ser222Glu
Trp208Val + Pro214Asn + Thr215Gly + Leu221Asp
Pro205Asn + Tyr213Met + Pro214Ser + Thr224Ser
Pro205Gly + Trp208Met + Ile209Leu + Tyr213Met
Pro205Gly + Thr217Glu + Tyr218Leu + Ala219His
Trp208Val + Tyr213Gly + Ser216Asp + Leu221Thr
Tyr210Gln + Thr212Pro + Tyr218Gly + Gly223Glu
Pro205Gly + Trp208Asp + Ile209His + Tyr210Ala
Trp208Ala + Ile209Leu + Tyr210Glu + Tyr213Cys
Gly206Asn + Trp208Leu + Tyr213Val + Ser216Asp
Ala204Pro + Pro205Gly + Thr217Glu + Leu221Ala
Trp208Asn + Thr212Asn + Tyr213His + Ser216Glu
Ala204His + Thr212Gly + Ala219Gln + Leu221Thr
Gly206Gln + Thr212Pro + Tyr213Met + Thr224Pro
Trp208Thr + Ile209Gln + Tyr213Ser + Ser222Asp
Ala204Asn + Ile209Asn + Tyr213Pro + Leu221Pro
Pro205Gly + Tyr210His + Tyr218Ile + Leu221Asn
Ala204Pro + Tyr210Cys + Pro214Gln + Thr215Pro
Pro205Ser + Tyr213Ser + Ser216Asp + Thr217Gly
Pro205Asn + Trp208Ser + Ala219Glu + Ser220Asp
Tyr210Ala + Tyr218Met + Ala219Glu + Ser220Asp
Ala204Gly + Pro205Gln + Pro214Asp + Thr215Asp
Tyr210Ser + Thr215Asn + Ser222Glu + Gly223Glu
Gly206Pro + Tyr213Val + Ser222Glu + Gly223Asp
Pro214Ser + Leu221Glu + Ser222Asp + Gly223Gln
Pro205Gln + Ser216Asp + Thr217Glu + Ala219Thr
```

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```
Trp208Leu + Thr212Gln + Ser220Glu + Leu221Glu
Gly206Gln + Ser220Asp + Leu221Asp + Gly223Asn
Thr217Asp + Tyr218Asp + Gly223Ser + Thr224Gly
Pro205Gln + Ile209Ala + Thr217Glu + Tyr218Glu
Pro214Gln + Thr215Asp + Ser216Glu + Thr224Pro
Ala204His + Ser207Glu + Tyr213Cys + Ser222Asp
Pro205Gly + Ser207Glu + Thr212Gln + Ser222Asp
Ser207Glu + Tyr210Ser + Tyr218Leu + Ser222Glu
Ser207Glu + Tyr218Cys + Ser222Glu + Thr224Pro
Pro205Ser + Trp208Glu + Leu221Glu + Ser222Glu
Trp208Glu + Ile209Asn + Thr217Gly + Ser222Glu
Ser216Glu + Thr217Asp + Tyr218Glu + Thr224Gly
Trp208Asp + Tyr218Leu + Ala219Thr + Leu221Glu
Ala204His + Thr212Gly + Thr215Glu + Thr217Glu
Pro214Asp + Ser216Asp + Ala219His + Gly223Asn
Tyr210Pro + Pro214Asp + Ser216Glu + Tyr218Ile
Pro205Gln + Tyr210Leu + Pro214Glu + Thr217Asp
Ala204Ser + Ser207Glu + Pro214Ser + Gly223Asp
Ser207Asp + Thr212Pro + Thr217Gly + Gly223Asp
Ser207Glu + Pro214Asn + Ser220Glu + Ser222Asp
Pro205Ser + Thr217Pro + Ala219Glu + Leu221Glu
Trp208Gln + Tyr210Glu + Thr212Asn + Leu221Glu
Trp208Gln + Thr215Pro + Ser220Glu + Ser222Glu
Gly206Pro + Thr217Gly + Ser220Asp + Ser222Glu
Gly206Gln + Tyr210Gly + Ser220Glu + Ser222Asp
Ser207Glu + Thr212Asn + Tyr218Pro + Leu221Asp
Ala204Asn + Ser207Glu + Trp208His + Leu221Asp
Tyr213Ile + Tyr218Leu + Ser222Asp + Thr224Glu
Gly206Gln + Tyr210Met + Ser222Asp + Thr224Glu
Ile209Ser + Tyr210His + Ser222Glu + Thr224Asp
Ala204Ser + Tyr218Asp + Ala219Pro + Ser220Glu
Gly206Gln + Thr217Ser + Tyr218Glu + Ser220Glu
Pro214Asp + Tyr218Asp + Gly223Ser + Thr224Gly
Gly206Gln + Tyr210Gly + Pro214Glu + Tyr218Glu
Trp208Asp + Thr217Gln + Ser220Asp + Gly223Asp
Ser207Glu + Tyr210Ala + Thr215Asn + Ser220Asp
Ser207Glu + Thr217Gly + Ser220Asp + Leu221Gln
Ser207Glu + Thr217Gly + Tyr218Asn + Ser220Asp
Pro205Asn + Ser207Asp + Thr212Gly + Ser220Asp
Ser207Glu + Thr212Asn + Ala219Gly + Ser220Asp
Gly206Pro + Ser207Glu + Trp208Asp + Thr224Asp
Ser207Asp + Trp208Thr + Tyr218Gln + Thr224Glu
Ala204Thr + Ser207Asp + Leu221Ala + Thr224Glu
Ser207Asp + Ala219Glu + Ser220Asp + Gly223Ser
Ser207Glu + Tyr210Glu + Thr215Gln + Thr224Asn
Ala204Gln + Tyr210Glu + Thr212Asn + Ser222Asp
```

TABLE 26

Loop 6 - Quintuple Substitution Variants

```
Gly206Asn + Tyr210Gly + Thr212Gln + Tyr218Ile +
                Thr224Gly
```

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```
Ala204Asn + Pro205Gly + Trp208Glu + Thr215Asn +
              Gly223Pro
Pro205Gln + Gly206Pro + Trp208Asn + Thr212Gln +
              Thr224Ser
Ala204Ser + Pro205Ser + Tyr213Ser + Thr215Ser +
              Ala219Gly
Ala204Gly + Gly206Gln + Thr212Pro + Ser220Asp +
              Leu221Ser
Pro214Ser + Thr215Gln + Tyr218Val + Ser222Asp +
              Thr224Asn
Ala204Pro + Ser207Asp + Tyr210Cys + Thr212Pro +
              Thr215Pro
Gly206Asn + Pro214Gln + Ala219Gly + Gly223Asn +
              Thr224Asp
Ala204Gln + Pro205Ser + Gly206Ser + Tyr210Gln +
              Ala219Asp
Gly206Pro + Ser207Asp + Trp208Met + Thr215Pro +
              Leu221Pro
Ala204Asn + Pro205Gln + Tyr210Cys + Thr212Asn +
              Leu221Ser
Ala204Asn + Gly206Gln + Trp208Thr + Ile209Ser +
              Ser222Asp
Pro205Gln + Tyr213Leu + Thr217Glu + Tyr218Met +
              Leu221Gln
Ala204Pro + Trp208Met + Ile209Val + Tyr210Leu +
              Ser216Glu
Trp208Ser + Ile209Ser + Tyr218His + Ala219His +
              Gly223Pro
Trp208Pro + Ile209Thr + Thr212Gly + Thr215Gly +
              Thr224Glu
Ile209Val + Pro214Ser + Ser216Asp + Tyr218Met +
              Leu221Met
Gly206Gln + Ile209Gln + Ser220Asp + Leu221Gly +
              Thr224Ser
Pro205Gln + Trp208Cys + Thr212Asn + Tyr213Cys +
              Pro214Asp
Pro205Gln + Trp208Gln + Thr217Asp + Gly223Ser +
              Thr224Gly
Ser207Glu + Thr212Ser + Thr217Gly + Tyr218Gln +
              Leu221His
Tyr210His + Thr212Ser + Leu221Glu + Gly223Ser +
              Thr224Pro
Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu +
              Thr224Gly
Gly206Ser + Trp208Phe + Ile209Ser + Tyr213Cys +
              Ser216Glu
Ala204Gln + Pro205Ser + Tyr210His + Tyr218Pro +
              Thr224Glu
Pro205Gln + Gly206Gln + Leu221His + Ser222Asp +
              Gly223Gln
Ala204Gly + Ile209His + Tyr210Met + Ser216Glu +
              Thr217Pro
Gly206Pro + Ile209Ala + Tyr210Gly + Leu221Asp +
```

```
                            Gly223Gln
      Gly206Asn + Tyr210Met + Thr215Glu + Gly223Asn +
                            Thr224Gly
      Gly206Gln + Ile209Pro + Ser216Asp + Leu221Cys +
                            Gly223Pro
      Trp208Thr + Tyr210Val + Thr212Ser + Pro214Asp +
                            Gly223Gln
      Thr212Gly + Pro214Gln + Thr215Ser + Leu221Gly +
                            Thr224Glu
      Ala204Thr + Thr212Ser + Thr217Ser + Gly223Asp +
                            Thr224Pro
      Pro205Ser + Ile209Pro + Tyr213Ile + Ala219Pro +
                            Ser222Glu
      Trp208Gly + Thr212Gln + Tyr218Gly + Ser222Glu +
                            Gly223Ser
      Gly206Pro + Ile209Pro + Tyr213Ser + Thr217Gly +
                            Tyr218Val
      Pro205Gln + Tyr213Cys + Pro214Gln + Ser220Glu +
                            Thr224Gly
      Gly206Asn + Thr212Gly + Tyr213Asn + Tyr218Pro +
                            Ser220Glu
      Ala204Pro + Pro205Ser + Thr212Gln + Thr217Asp +
                            Thr224Ser
      Pro205Asn + Trp208Ile + Thr212Gln + Pro214Ser +
                            Thr217Gly
      Ile209Cys + Thr217Gly + Ser220Asp + Leu221Gln +
                            Gly223Gln
      Ile209Thr + Thr215Gly + Ser216Glu + Tyr218Leu +
                            Ala219Pro
      Ile209Met + Pro214Gly + Thr217Gly + Tyr218Cys +
                            Leu221Met
      Pro205Gly + Pro214Asn + Tyr218Ile + Ala219Gly +
                            Ser222Asp
      Tyr210Thr + Thr212Ser + Pro214Ser + Leu221Asn +
                            Ser222Asp
      Gly206Asn + Tyr210Ile + Pro214Gln + Ala219Asn +
                            Leu221Val
      Pro205Asn + Thr215Ser + Ala219Ser + Leu221Ala +
                            Thr224Asp
      Gly206Pro + Pro214Asn + Thr215Gln + Tyr218Ser +
                            Leu221Thr
      Ala204Pro + Gly206Gln + Ile209His + Thr212Gly +
                            Gly223Glu
      Pro205Gln + Tyr213Gly + Tyr218Ala + Gly223Glu +
                            Thr224Pro
      Pro205Ser + Pro214Gly + Thr217Pro + Ala219Thr +
                            Leu221Glu
      Ala204Pro + Pro205Asn + Ser207Asp + Tyr210Ala +
                            Pro214Ser
      Gly206Ser + Tyr213Thr + Ala219Gln + Ser220Glu +
                            Gly223Ser
      Pro205Gly + Thr212Asn + Thr215Pro + Ser216Glu +
                            Leu221Asn
```

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Ser207Glu + Ile209Ala + Thr212Asn + Thr215Gln +
            Tyr218Met
Tyr213His + Thr215Gln + Thr217Asn + Ala219Glu +
            Leu221Ser
Gly206Gln + Tyr213Asn + Thr215Ser + Tyr218Ala +
            Leu221Ala
Trp208Met + Tyr210Met + Tyr218Gln + Ser220Glu +
            Thr224Ser
Ala204Thr + Pro205Ser + Ile209Leu + Thr217Asn +
            Thr224Pro
Pro205Gly + Ile209Leu + Thr215Asn + Ser216Glu +
            Gly223Ser
Ile209Ser + Tyr213Leu + Thr217Asp + Tyr218Asn +
            Ala219His
Ala204Ser + Pro205Ser + Ser207Glu + Leu221Val +
            Gly223Pro
Ala204His + Thr212Gly + Ala219Gln + Leu221Thr +
            Gly223Glu
Pro205Ser + Gly206Gln + Thr212Pro + Gly223Pro +
            Thr224Pro
Ala204Asn + Trp208Thr + Tyr213Pro + Leu221Pro +
            Ser222Asp
Pro205Gly + Ile209Met + Tyr218Met + Leu221Val +
            Gly223Asn
Thr212Gly + Tyr218Ile + Ala219Asn + Ser220Glu +
            Thr224Pro
Ala204Gln + Pro205Gln + Gly206Gln + Ile209Met +
            Ser216Asp
Ala204Thr + Thr212Gly + Ala219Asn + Gly223Asp +
            Thr224Asn
Ala204Pro + Trp208His + Tyr213Leu + Thr217Ser +
            Ser222Glu
Pro205Ser + Ser207Asp + Ile209Asn + Tyr213Met +
            Thr217Gln
Gly206Pro + Trp208Ser + Thr212Gly + Tyr213Cys +
            Gly223Glu
Ala204Asn + Gly206Pro + Trp208Val + Pro214Asn +
            Tyr218Glu
Ile209Gly + Tyr210Ala + Tyr218Pro + Gly223Ser +
            Thr224Ser
Pro205Gln + Tyr213His + Tyr218Met + Ser220Glu +
            Leu221Gly
Ala204Asn + Tyr210Cys + Thr212Gln + Tyr218Leu +
            Ser222Asp
Gly206Ser + Ile209Leu + Thr212Gly + Pro214Gln +
            Thr215Glu
Ser207Asp + Pro214Ser + Thr215Asn + Thr217Asn +
            Leu221Ser
Pro205Gly + Trp208Thr + Thr217Gly + Ser220Glu +
            Gly223Ser
Gly206Ser + Trp208Glu + Tyr210Gln + Thr217Gln +
            Leu221Pro
Gly206Gln + Ser207Glu + Thr212Asn + Pro214Ser +

```
                          Leu221Ala
    Ala204Ser + Trp208Leu + Ile209Asn + Thr215Asp +
                          Thr224Asn
    Gly206Ser + Ile209Pro + Thr217Ser + Ser220Glu +
                          Gly223Gln
    Pro205Gln + Trp208Pro + Thr212Pro + Pro214Glu +
                          Ala219Ser
    Gly206Pro + Ser207Glu + Tyr210Met + Thr212Gly +
                          Tyr213Leu
    Ala204His + Tyr210Pro + Tyr213Ala + Thr217Glu +
                          Thr224Ser
    Trp208Cys + Ile209Met + Tyr210Ala + Tyr213Met +
                          Ala219Asn
    Trp208Thr + Tyr210Ala + Tyr218Met + Ala219Glu +
                          Ser220Asp
    Trp208Asn + Ile209Pro + Ala219Asp + Ser220Asp +
                          Gly223Ser
    Pro205Ser + Tyr213Thr + Pro214Asp + Thr215Asp +
                          Tyr218Val
    Ile209Ser + Tyr213Pro + Leu221Met + Ser222Asp +
                          Gly223Asp
    Ala204Thr + Thr217Gln + Tyr218Ile + Ser222Glu +
                          Gly223Asp
    Pro205Ser + Tyr210Ala + Thr215Asn + Ser222Glu +
                          Gly223Glu
    Tyr210Pro + Thr212Pro + Leu221Asn + Ser222Asp +
                          Gly223Asp
    Trp208Pro + Ile209Pro + Tyr213Ile + Ser222Asp +
                          Gly223Asp
    Gly206Gln + Tyr213Gly + Thr217Gly + Ser222Glu +
                          Gly223Glu
    Trp208Ser + Ile209Pro + Thr212Pro + Ser216Glu +
                          Thr217Asp
    Gly206Asn + Tyr218Met + Ala219His + Ser220Glu +
                          Leu221Asp
    Ile209Ala + Thr215Glu + Ser216Glu + Gly223Gln +
                          Thr224Asn
    Ile209Gly + Pro214Ser + Thr215Glu + Ser216Asp +
                          Tyr218Ile
    Ala204Ser + Trp208Val + Ile209Ser + Thr215Glu +
                          Ser216Asp
    Thr212Pro + Thr215Asp + Ser216Glu + Thr217Glu +
                          Thr224Ser
    Ala204His + Ser207Glu + Trp208His + Tyr210His +
                          Ser222Asp
    Ser207Glu + Tyr213Asn + Leu221Thr + Ser222Asp +
                          Thr224Gly
    Gly206Ser + Ser207Glu + Trp208Pro + Ala219Thr +
                          Ser222Asp
    Pro205Gly + Ser207Glu + Thr212Gln + Tyr213Gln +
                          Ser222Asp
    Pro205Ser + Ser207Glu + Tyr213His + Thr217Pro +
                          Ser222Glu
```

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Gly206Pro + Ser207Asp + Tyr213Ile + Tyr218Asn +
                        Ser222Glu
Gly206Pro + Trp208Glu + Tyr210Cys + Pro214Gln +
                        Ser220Asp
Tyr210Glu + Tyr213Gly + Tyr218Leu + Ala219Asp +
                        Thr224Ser
Gly206Pro + Ser207Asp + Leu221Asp + Ser222Glu +
                        Thr224Gln
Tyr213Ser + Pro214Ser + Thr215Glu + Thr217Asp +
                        Tyr218Ala
Ala204Asn + Tyr210Gln + Thr212Asn + Pro214Glu +
                        Ser216Asp
Pro205Gln + Gly206Pro + Pro214Glu + Ser216Asp +
                        Thr224Ser
Ser207Asp + Thr212Pro + Thr215Gly + Thr217Gly +
                        Gly223Asp
Ser207Glu + Tyr210Val + Pro214Asn + Leu221Thr +
                        Gly223Glu
Ser207Asp + Ile209Thr + Tyr210Met + Thr212Pro +
                        Gly223Asp
Ser207Glu + Tyr210Ser + Tyr218Leu + Ser220Asp +
                        Ser222Glu
Ser207Asp + Thr217Gln + Ser220Asp + Ser222Asp +
                        Thr224Asn
Trp208Thr + Thr217Glu + Tyr218Ser + Ala219Glu +
                        Leu221Cys
Pro205Gly + Thr212Ser + Ala219Gln + Ser220Glu +
                        Ser222Glu
Gly206Pro + Trp208His + Tyr210Gly + Ser220Glu +
                        Ser222Asp
Ala204Gln + Gly206Ser + Trp208Gln + Ser220Glu +
                        Ser222Glu
Ala204Ser + Tyr213Thr + Ser220Asp + Leu221Met +
                        Ser222Asp
Thr212Gly + Thr215Asp + Ser216Asp + Thr217Gly +
                        Tyr218Asp
Ala204Asn + Tyr210Glu + Tyr213Ala + Ser220Glu +
                        Ser222Glu
Ala204His + Gly206Pro + Ser216Glu + Tyr218Asp +
                        Leu221Ser
Gly206Pro + Ile209Ser + Tyr210His + Ser222Glu +
                        Thr224Asp
Trp208Met + Tyr218Leu + Ala219Pro + Ser222Glu +
                        Thr224Glu
Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Asp +
                        Ser220Glu
Ala204Ser + Tyr213Gln + Tyr218Asp + Ala219Pro +
                        Ser220Glu
Thr212Asn + Tyr213Gln + Thr217Ser + Tyr218Glu +
                        Ser220Glu
Pro214Ser + Leu221Glu + Ser222Asp + Gly223Ser +
                        Thr224Asp
Pro205Gly + Tyr210Cys + Ala219Asp + Ser220Glu +

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```
                        Ser222Asp
Gly206Gln + Tyr210Gly + Pro214Glu + Thr217Pro +
                        Tyr218Glu
Trp208Asp + Pro214Ser + Thr217Gln + Ser220Asp +
                        Gly223Asp
Ser207Glu + Tyr210Ala + Thr215Asn + Ser220Asp +
                        Leu221Ala
Ser207Asp + Trp208Cys + Ile209Ala + Thr215Asn +
                        Ser220Glu
Ser207Asp + Tyr210Met + Tyr218Asn + Ser220Glu +
                        Leu221Cys
Ser207Glu + Tyr210Pro + Thr215Gln + Tyr218Cys +
                        Ser220Glu
Ser207Glu + Thr212Ser + Pro214Gln + Ser220Asp +
                        Leu221Pro
Pro205Asn + Ser207Asp + Thr215Pro + Ser220Asp +
                        Leu221Thr
Trp208Glu + Ile209Asn + Thr217Gly + Ala219Asp +
                        Ser222Glu
Pro205Asn + Ser207Glu + Tyr213Leu + Ser220Asp +
                        Gly223Glu
Ala204Ser + Pro205Gly + Ser207Asp + Thr212Ser +
                        Thr224Asp
Ala204Pro + Gly206Gln + Ser207Glu + Tyr218Asn +
                        Thr224Glu
Gly206Ser + Ser207Glu + Tyr210Met + Tyr213Cys +
                        Thr224Asp
Pro205Asn + Ser207Glu + Ile209Val + Tyr213Pro +
                        Thr224Glu
Ser207Asp + Ile209Val + Tyr213Ala + Gly223Gln +
                        Thr224Glu
Pro205Asn + Ser207Glu + Ala219Glu + Ser220Glu +
                        Gly223Gln
Gly206Asn + Ser207Glu + Trp208Asp + Ala219Asp +
                        Thr224Pro
Ala204Gly + Gly206Ser + Ser207Asp + Tyr210Asp +
                        Leu221Gly
Ala204Pro + Ser207Glu + Ile209Thr + Tyr210Glu +
                        Tyr218Leu
Ile209Thr + Tyr213Gly + Ala219Asp + Ser222Glu +
                        Gly223Asp
Pro205Gln + Tyr213Gln + Tyr218Glu + Leu221Glu +
                        Ser222Asp
Gly206Gln + Ser207Glu + Thr212Asn + Ser220Glu +
                        Thr224Glu
Ser207Glu + Thr217Gly + Tyr218Asn + Ser220Asp +
                        Thr224Glu
Ala204Thr + Tyr210Cys + Ser220Glu + Leu221Ser +
                        Gly223Glu
Ile209Pro + Ser216Glu + Ala219Glu + Ser220Asp +
                        Gly223Asn
Gly206Gln + Tyr210Met + Thr212Pro + Ala219Glu +
                        Ser222Asp
```

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Pro205Asn + Tyr210Glu + Thr217Asp + Tyr218Ala +
                    Thr224Pro
Ala204Gly + Trp208Gln + Thr212Gln + Ser216Asp +
                    Ala219Glu
Ala204Asn + Trp208Ala + Ser216Asp + Ala219Glu +
                    Thr224Gly
Ala204Asn + Tyr210Asp + Thr215Glu + Thr217Glu +
                    Ala219Gly
Trp208Leu + Ile209Gly + Thr215Asp + Thr217Glu +
                    Ser220Asp
Thr212Pro + Tyr213Leu + Ser216Glu + Tyr218Glu +
                    Leu221Glu
Thr217Asp + Ala219Glu + Leu221Asn + Ser222Glu +
                    Thr224Ser
Pro205Ser + Trp208Glu + Tyr218Glu + Leu221Gly +
                    Ser222Glu
Ser207Glu + Thr215Pro + Thr217Asp + Ser220Glu +
                    Gly223Pro
Thr217Asp + Ala219Asn + Leu221Glu + Ser222Glu +
                    Gly223Asn
Trp208Tyr + Ile209Asn + Thr217Glu + Leu221Glu +
                    Ser222Asp
Ala204Gly + Tyr210Glu + Pro214Glu + Ser216Glu +
                    Thr217Asn
Pro214Asp + Thr215Gln + Ser216Glu + Ser220Glu +
                    Leu221Met
Ala204Gln + Tyr210Glu + Thr212Asn + Thr217Glu +
                    Ser222Asp
Trp208Leu + Ile209Val + Thr215Asp + Thr217Glu +
                    Leu221Glu
Gly206Pro + Ile209Val + Tyr213Pro + Thr215Asp +
                    Ala219Glu
Pro205Asn + Ser207Glu + Tyr218Asp + Ser222Asp +
                    Thr224Gly
Gly206Ser + Tyr210Asp + Pro214Asp + Thr215Pro +
                    Leu221Asp
Tyr210Ser + Pro214Asp + Tyr218Cys + Ser220Glu +
                    Ser222Glu
Ser207Asp + Ile209Ser + Thr212Ser + Pro214Asp +
                    Thr215Asp

TABLE 27

Loop 6 - Sextuple Substitution Variants

Gly206Asn + Tyr210Gly + Thr212Gln + Tyr213Gly +
    Tyr218Ile + Thr224Gly
Pro205Asn + Gly206Ser + Tyr213Gly + Ser216Asp +
    Thr217Ser + Thr224Asn
Ala204Ser + Pro205Ser + Tyr213Ser + Tyr218His +
    Ala219Gln + Leu221Asn
Ser207Glu + Thr212Gln + Thr215Pro + Tyr218Gln +
    Leu221Met + Gly223Gln

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Pro205Ser + Ser207Asp + Tyr210Val + Tyr213Leu +
　　Leu221Gln + Thr224Gly
Ala204Ser + Trp208Cys + Ile209His + Thr215Glu +
　　Thr217Pro + Gly223Ser
Ala204Gly + Ile209Asn + Tyr210Ile + Ser216Asp +
　　Ala219Asn + Leu221Met
Ala204Gly + Pro205Gln + Pro214Glu + Thr215Ser +
　　Tyr218Asn + Thr224Ser
Gly206Asn + Trp208Cys + Thr212Gly + Tyr213Gly +
　　Ser216Asp + Leu221His
Trp208Pro + Ile209Gly + Thr215Ser + Ala219Thr +
　　Leu221Thr + Thr224Asp
Pro205Gly + Gly206Gln + Ile209Gly + Thr212Asn +
　　Pro214Asn + Ser220Glu
Pro205Gly + Trp208Leu + Thr217Gln + Leu221Gln +
　　Gly223Gln + Thr224Glu
Ala204Gly + Gly206Asn + Tyr210Cys + Tyr213Thr +
　　Gly223Asp + Thr224Asn
Gly206Asn + Trp208His + Ile209Gly + Thr217Gly +
　　Leu221Val + Thr224Pro
Ala204Thr + Thr212Gln + Tyr213Gln + Thr217Ser +
　　Ser222Asp + Thr224Pro
Trp208Gly + Thr212Gln + Tyr213Leu + Tyr218Gly +
　　Ser222Glu + Gly223Ser
Pro205Ser + Trp208Ser + Tyr213Asn + Tyr218Gln +
　　Ser222Asp + Thr224Gln
Ala204His + Pro205Gly + Ile209Asn + Tyr213Gln +
　　Thr215Ser + Thr217Ser
Ala204Thr + Gly206Asn + Trp208Leu + Thr215Ser +
　　Ser216Asp + Ala219His
Gly206Ser + Thr212Gln + Tyr213Val + Tyr218Cys +
　　Ser220Glu + Thr224Gln
Gly206Asn + Trp208Ile + Ile209Ser + Tyr218Gln +
　　Gly223Glu + Thr224Gly
Gly206Asn + Ser207Glu + Ile209Cys + Thr212Ser +
　　Thr217Pro + Ala219His
Ala204Asn + Tyr210Cys + Tyr213Asn + Tyr218Leu +
　　Leu221His + Ser222Glu
Gly206Pro + Tyr213Asn + Pro214Asn + Thr215Gln +
　　Tyr218Ser + Leu221Thr
Ala204Pro + Pro205Asn + Gly206Gln + Ile209His +
　　Thr212Gly + Gly223Glu
Pro205Gln + Tyr213Gly + Thr215Gly + Tyr218Ala +
　　Gly223Glu + Thr224Pro
Ala204His + Ser207Glu + Trp208Gly + Ile209His +
　　Tyr213Cys + Thr215Ser
Gly206Ser + Trp208His + Tyr213Thr + Ala219Gln +
　　Ser220Glu + Gly223Ser
Tyr213His + Thr215Gln + Thr217Asn + Tyr218Ala +
　　Ala219Glu + Leu221Ser
Ala204Asn + Pro205Ser + Gly206Asn + Ile209Leu +
　　Thr215Asp + Thr224Pro
Pro205Ser + Ile209Gly + Tyr213Leu + Thr217Asp +

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```
Tyr218Asn + Ala219His
Ala204Asn + Pro205Gln + Ser207Asp + Tyr210Val +
    Thr212Gly + Leu221Thr
Ala204Asn + Trp208Thr + Ile209Gln + Tyr213Pro +
    Leu221Pro + Ser222Asp
Ala204Ser + Gly206Ser + Tyr213Gly + Pro214Gln +
    Tyr218Val + Ser222Asp
Ala204Pro + Tyr210Cys + Tyr213Ser + Pro214Gln +
    Thr215Pro + Thr217Gly
Thr212Ser + Pro214Asp + Thr217Gly + Ala219Thr +
    Leu221Pro + Thr224Gln
Pro205Gln + Gly206Gln + Ser207Glu + Tyr210Gly +
    Thr212Pro + Tyr218Gly
Pro205Gln + Gly206Pro + Trp208Asn + Ile209Met +
    Tyr213Ser + Ala219Asp
Ala204Asn + Ser207Glu + Trp208Ala + Tyr213Asn +
    Leu221Thr + Thr224Gly
Ala204Pro + Trp208His + Ile209Asn + Tyr213Leu +
    Thr217Ser + Ser222Glu
Gly206Pro + Trp208Ser + Tyr210Val + Thr212Gly +
    Tyr213Cys + Ala219Asp
Pro205Gly + Gly206Gln + Ile209Cys + Tyr210His +
    Thr215Gln + Ala219Thr
Trp208Gly + Ile209Ser + Thr212Gln + Tyr218Met +
    Leu221Ser + Gly223Ser
Pro205Asn + Ile209Asn + Thr212Asn + Thr217Glu +
    Gly223Pro + Thr224Ser
Pro205Gly + Trp208Thr + Thr212Ser + Ser216Glu +
    Thr217Gly + Gly223Ser
Gly206Ser + Trp208Glu + Tyr210Gln + Thr215Gly +
    Thr217Gln + Leu221Pro
Gly206Ser + Ser207Glu + Trp208Leu + Thr212Asn +
    Leu221Ala + Thr224Asn
Ala204Asn + Pro205Gln + Gly206Gln + Trp208Ile +
    Ser216Asp + Tyr218Cys
Ala204Thr + Tyr210Cys + Tyr213Ser + Thr215Ser +
    Ser216Asp + Ala219His
Trp208Leu + Ile209Asn + Thr212Gly + Thr215Ser +
    Tyr218Pro + Leu221Gly
Pro205Gly + Gly206Gln + Tyr218Gly + Ala219Asn +
    Leu221Gln + Thr224Ser
Pro205Gln + Trp208Val + Tyr210Pro + Thr215Gly +
    Ala219Ser + Thr224Glu
Pro205Gly + Trp208Met + Ile209Ser + Thr212Asn +
    Tyr218Ala + Ala219Thr
Thr212Gly + Tyr213Asn + Pro214Ser + Ser216Glu +
    Thr217Pro + Leu221Thr
Ala204Gly + Trp208Pro + Ile209Pro + Tyr213Gln +
    Tyr218Ser + Ser220Glu
Ala204Gly + Gly206Asn + Ile209Met + Thr212Pro +
    Ala219Glu + Leu221Met
Pro205Asn + Gly206Pro + Trp208Leu + Tyr210Pro +
    Thr217Gln + Tyr218Glu
```

Pro205Asn + Gly206Gln + Tyr213Val + Ser220Asp +
  Gly223Ser + Thr224Asn
Ala204Thr + Pro205Gly + Gly206Gln + Pro214Gln +
  Leu221Val + Gly223Gln
Pro205Ser + Ile209Gln + Thr212Pro + Ala219Gly +
  Ser220Glu + Gly223Gln
Ala204Gly + Gly206Gln + Ile209Leu + Thr212Asn +
  Pro214Gln + Thr215Ser
Gly206Asn + Trp208Ser + Thr212Ser + Pro214Asn +
  Ser220Glu + Gly223Ser
Ala204His + Tyr213Leu + Pro214Asn + Thr217Pro +
  Ser220Glu + Gly223Gln
Gly206Pro + Ile209Cys + Pro214Gln + Thr217Ser +
  Tyr218Cys + Ala219Gln
Ala204Thr + Tyr210Ala + Thr215Gly + Thr217Gln +
  Ala219Pro + Ser220Asp
Ser207Asp + Ile209Thr + Thr212Gly + Thr217Pro +
  Ala219Gly + Thr224Pro
Ala204Gly + Ser207Glu + Thr212Asn + Pro214Ser +
  Tyr218Ser + Ala219Ser
Pro205Gln + Gly206Gln + Thr212Ser + Tyr213Ala +
  Thr215Ser + Gly223Glu
Pro205Ser + Tyr210Ala + Pro214Ser + Thr215Asn +
  Ser222Asp + Gly223Glu
Ile209Pro + Tyr210Pro + Thr212Pro + Leu221Asn +
  Ser222Asp + Gly223Asp
Pro205Ser + Thr212Ser + Tyr213Ile + Ala219Pro +
  Ser222Glu + Gly223Asp
Ala204Asn + Ile209Cys + Tyr213Cys + Tyr218Ala +
  Leu221Asp + Ser222Glu
Ile209Val + Thr212Asn + Tyr213Met + Thr217Asn +
  Gly223Asp + Thr224Asp
Pro205Gly + Ile209Asn + Thr212Asn + Ser216Glu +
  Thr217Glu + Tyr218Gln
Ala204His + Trp208Ser + Thr212Pro + Tyr213Pro +
  Ser216Glu + Thr217Asp
Pro205Ser + Ile209Ala + Thr212Gly + Ser216Asp +
  Thr217Glu + Ala219Thr
Pro205Ser + Trp208Met + Pro214Asn + Ala219Gln +
  Ser220Glu + Leu221Asp
Ala204Pro + Gly206Ser + Tyr213Ile + Ser220Glu +
  Leu221Asp + Gly223Ser
Gly206Pro + Tyr210Gly + Tyr213His + Thr217Glu +
  Tyr218Asp + Leu221Asn
Thr212Pro + Tyr213Ser + Thr215Asp + Ser216Asp +
  Tyr218Pro + Gly223Gln
Ser207Glu + Ile209Asn + Thr212Ser + Thr217Gly +
  Ser222Glu + Thr224Gln
Pro205Gly + Ser207Glu + Tyr210Ser + Pro214Gln +
  Ser222Asp + Gly223Asn
Gly206Pro + Ser207Asp + Trp208Ser + Pro214Ser +
  Leu221Asn + Ser222Asp
Gly206Gln + Ser207Asp + Ile209Cys + Tyr210Met +

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    Thr217Asn + Ser222Glu
Gly206Pro + Ser207Glu + Ile209Pro + Tyr210Ile +
    Leu221Asn + Ser222Glu
Pro205Ser + Ser207Glu + Trp208His + Thr215Pro +
    Leu221Asn + Ser222Glu
Pro205Asn + Ser207Asp + Thr215Gln + Tyr218Ile +
    Leu221Gly + Ser222Glu
Ala204Pro + Ser207Asp + Tyr210Cys + Thr212Pro +
    Thr215Pro + Ser222Glu
Pro205Gly + Pro214Asp + Thr215Glu + Ser216Asp +
    Thr217Pro + Gly223Gln
Ile209Thr + Thr217Gly + Ala219Glu + Ser220Asp +
    Leu221Glu + Gly223Ser
Ala204Gln + Ser207Asp + Ile209Thr + Tyr218Asn +
    Ser222Glu + Gly223Asp
Pro205Ser + Ser207Glu + Pro214Gly + Thr215Asn +
    Ser222Asp + Gly223Glu
Pro205Asn + Gly206Pro + Trp208Asp + Thr212Gln +
    Ser222Asp + Gly223Ser
Gly206Gln + Pro214Asp + Thr215Asp + Thr217Asp +
    Gly223Ser + Thr224Gly
Ala204His + Ile209Thr + Ser216Asp + Thr217Asp +
    Tyr218Asp + Leu221Ser
Thr212Asn + Pro214Gln + Tyr218Met + Ser222Glu +
    Gly223Asp + Thr224Glu
Ala204Gln + Gly206Pro + Tyr210Glu + Pro214Gly +
    Ala219Asp + Leu221Gln
Ala204Gly + Pro205Gln + Trp208Asp + Ile209Gln +
    Pro214Gly + Leu221Glu
Ala204Asn + Gly206Gln + Ser207Glu + Trp208His +
    Leu221Asp + Ser222Asp
Trp208Gly + Tyr213Ser + Pro214Ser + Thr215Glu +
    Thr217Asp + Tyr218Ala
Ala204Asn + Pro205Ser + Tyr210Pro + Pro214Asp +
    Ser216Glu + Tyr218Ile
Gly206Gln + Ile209Leu + Thr212Gly + Pro214Asp +
    Ser216Glu + Leu221His
Pro205Gln + Trp208Glu + Tyr210Asp + Thr212Gln +
    Tyr213Asn + Thr215Ser
Ser207Asp + Thr212Pro + Thr215Gly + Thr217Gln +
    Leu221Met + Gly223Asp
Ala204Asn + Pro205Gln + Ser207Glu + Trp208Met +
    Ala219Pro + Gly223Asp
Ala204Thr + Ser207Asp + Thr212Gly + Ala219Asn +
    Gly223Asp + Thr224Asn
Ser207Asp + Pro214Asn + Thr215Gly + Tyr218Ala +
    Leu221Ser + Gly223Asp
Ser207Glu + Thr212Asn + Tyr213Gln + Tyr218Pro +
    Ser220Glu + Leu221Asp
Ala204Asn + Gly206Ser + Ser207Asp + Tyr210Thr +
    Ser220Asp + Leu221Asp
Gly206Ser + Thr212Asn + Thr215Pro + Tyr218Val +
    Leu221Asp + Gly223Asp
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Trp208Met + Tyr210Thr + Thr212Asn + Ala219Gln +
  Leu221Asp + Gly223Asp
Gly206Ser + Ser207Glu + Pro214Ser + Ala219Asn +
  Ser220Glu + Ser222Glu
Ala204His + Ser207Glu + Ala219Ser + Ser220Glu +
  Leu221His + Ser222Asp
Pro205Ser + Ser207Glu + Tyr213His + Thr217Pro +
  Ser220Glu + Ser222Glu
Ser207Glu + Ile209Met + Tyr210Gly + Tyr213Leu +
  Ser220Glu + Ser222Asp
Ser207Asp + Tyr213Pro + Pro214Gln + Ser220Glu +
  Ser222Glu + Gly223Pro
Gly206Ser + Trp208Gln + Tyr210Glu + Thr212Asn +
  Thr215Gly + Leu221Glu
Pro205Gly + Thr212Ser + Tyr218Leu + Ala219Gln +
  Ser220Glu + Ser222Glu
Trp208Phe + Thr212Asn + Pro214Ser + Ser220Glu +
  Ser222Asp + Gly223Gln
Ala204Gly + Gly206Pro + Tyr210Ser + Tyr218Cys +
  Ser220Glu + Ser222Asp
Pro205Ser + Trp208Leu + Tyr213Ala + Thr217Ser +
  Ser220Asp + Ser222Asp
Pro205Gly + Trp208His + Tyr210His + Ser220Glu +
  Leu221Gln + Ser222Asp
Pro205Gly + Thr212Gln + Ala219Thr + Ser220Glu +
  Ser222Asp + Gly223Pro
Ala204Ser + Gly206Pro + Tyr210Gly + Ser220Glu +
  Leu221Ser + Ser222Asp
Ile209Ser + Tyr210Ser + Thr212Asn + Tyr213Cys +
  Ser220Asp + Ser222Glu
Ala204Asn + Pro205Gly + Ser207Asp + Ile209Ser +
  Tyr213Val + Leu221Glu
Ala204Asn + Gly206Pro + Thr215Glu + Ser216Glu +
  Thr217Asn + Tyr218Glu
Gly206Pro + Ile209Ser + Tyr210His + Thr217Gly +
  Ser222Glu + Thr224Asp
Gly206Gln + Thr217Gly + Ser220Asp + Ser222Glu +
  Gly223Glu + Thr224Ser
Gly206Gln + Trp208Gln + Ile209Gln + Ser216Asp +
  Tyr218Asp + Ala219Glu
Gly206Asn + Thr212Pro + Pro214Ser + Thr217Glu +
  Tyr218Glu + Ser220Glu
Gly206Gln + Trp208Ser + Tyr210Asp + Pro214Gln +
  Thr217Glu + Ala219Asp
Gly206Ser + Ser207Glu + Trp208Pro + Tyr210Asp +
  Ala219Thr + Ser222Asp
Trp208Gly + Tyr210His + Thr212Ser + Pro214Glu +
  Tyr218Asp + Leu221Ile
Ala204Gln + Gly206Gln + Tyr210Gly + Pro214Glu +
  Thr217Pro + Tyr218Glu
Ala204His + Pro214Glu + Thr215Gln + Thr217Gln +
  Tyr218Glu + Leu221Pro
Ser207Asp + Ile209Cys + Thr215Pro + Tyr218His +

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     Ala219Thr + Ser220Glu
Pro205Asn + Ser207Glu + Trp208Tyr + Ile209Asn +
     Thr217Gln + Ser220Glu
Ser207Asp + Pro214Ser + Thr215Asn + Thr217Asn +
     Ser220Glu + Leu221Ser
Pro205Gly + Ser207Asp + Tyr210Ser + Ser220Asp +
     Gly223Pro + Thr224Ser
Gly206Gln + Ser207Glu + Ile209Gln + Ser220Asp +
     Leu221Ala + Thr224Ser
Ala204Ser + Pro205Gly + Ser207Asp + Thr212Ser +
     Thr215Gln + Ser220Glu
Pro205Ser + Ser207Asp + Thr217Gln + Ala219His +
     Ser220Glu + Gly223Glu
Ser207Glu + Thr215Gln + Tyr218Val + Ser220Asp +
     Gly223Glu + Thr224Asn
Pro205Gly + Ile209Pro + Tyr210Asp + Ala219Asp +
     Ser222Asp + Gly223Ser
Gly206Asn + Tyr210Gln + Pro214Asn + Thr215Glu +
     Tyr218Glu + Leu221Ala
Ile209Asn + Tyr210Asp + Thr212Gln + Ser222Glu +
     Gly223Pro + Thr224Asn
Ala204Gly + Gly206Gln + Ser220Asp + Leu221Asp +
     Gly223Asn + Thr224Glu
Gly206Pro + Thr212Pro + Thr217Pro + Tyr218Asn +
     Leu221Glu + Thr224Glu
Ile209His + Tyr210Leu + Tyr213Leu + Pro214Asp +
     Thr217Asp + Ser220Glu
Ile209Asn + Tyr210Ile + Thr212Asn + Thr215Gly +
     Ser220Asp + Gly223Glu
Gly206Gln + Tyr210Ala + Thr215Asn + Ser216Glu +
     Thr217Asp + Ser220Asp
Trp208Thr + Tyr213Pro + Tyr218His + Ala219Glu +
     Leu221Cys + Ser222Asp
Ala204Gly + Trp208Gln + Thr212Gln + Ser216Asp +
     Ala219Glu + Gly223Ser
Gly206Ser + Pro214Gly + Ser216Asp + Tyr218Pro +
     Ala219Glu + Leu221Pro
Pro205Asn + Tyr210Asn + Thr217Glu + Ala219Gln +
     Ser220Glu + Gly223Gln
Ala204Thr + Tyr213Ala + Pro214Gln + Thr215Gln +
     Thr217Asp + Ser220Glu
Trp208Gln + Tyr210His + Thr217Asp + Tyr218Met +
     Ser220Asp + Thr224Ser
Ala204Asn + Gly206Pro + Tyr210Asp + Pro214Glu +
     Ser220Glu + Thr224Asn
Tyr213Ile + Ser216Glu + Tyr218His + Ala219Glu +
     Leu221Asp + Thr224Ser
Pro205Ser + Trp208Glu + Tyr218Glu + Leu221Gly +
     Ser222Glu + Thr224Gly
Ser207Asp + Tyr213Met + Pro214Gly + Thr217Gln +
     Tyr218Asp + Ala219Glu
Trp208Phe + Ala219Asp + Leu221Ala + Ser222Asp +
     Gly223Asn + Thr224Glu
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Gly206Pro + Ile209His + Pro214Glu + Ser220Asp +
    Leu221Glu + Thr224Pro
Trp208Tyr + Ile209Asn + Thr212Asn + Thr217Glu +
    Leu221Glu + Ser222Asp
Pro205Gly + Ser216Glu + Thr217Glu + Ala219Gln +
    Leu221Glu + Gly223Gln
Pro214Asn + Thr217Pro + Ala219Asp + Ser220Asp +
    Leu221Gln + Thr224Asp
Ala204His + Gly206Asn + Ile209His + Pro214Glu +
    Ser216Asp + Ser220Glu
Ser207Asp + Tyr210Glu + Thr217Asp + Tyr218Ala +
    Leu221Ile + Thr224Pro
Thr212Gly + Tyr213Ala + Pro214Glu + Leu221Glu +
    Ser222Asp + Thr224Ser
Pro205Gln + Gly206Asn + Thr212Gln + Tyr218Glu +
    Ser222Asp + Gly223Asp
Pro205Gln + Thr212Gly + Tyr213Ala + Thr217Glu +
    Tyr218Glu + Ser222Glu
Ile209Gln + Tyr213Ala + Pro214Glu + Ala219Asp +
    Ser222Asp + Thr224Pro
Ala204Gly + Tyr210Pro + Pro214Glu + Ala219Glu +
    Leu221Asn + Ser222Asp
Gly206Pro + Thr212Asn + Tyr213Thr + Pro214Asp +
    Ser220Asp + Ser222Glu
Ala204Thr + Tyr213Ser + Pro214Asp + Ala219Asn +
    Ser220Asp + Ser222Glu
Ala204His + Gly206Asn + Tyr210Pro + Pro214Glu +
    Ser220Glu + Ser222Glu
Ser207Asp + Trp208Met + Pro214Glu + Thr215Asp +
    Thr217Ser + Gly223Pro
Trp208Cys + Ile209Ser + Pro214Glu + Ser222Asp +
    Gly223Asp + Thr224Gly
Pro205Asn + Thr212Asn + Tyr213Leu + Thr215Asp +
    Ser222Asp + Gly223Glu

TABLE 28

Loop 6 - Heptuple Substitution Mutation Variants
Gly206Asn + Tyr210Gly + Thr212Gln + Thr215Gln +   Tyr218Ile + Leu221Ile + Thr224Gly
Ala204Ser + Pro205Ser + Ile209Met + Tyr213Ser +   Tyr218His + Ala219Gln + Leu221Asn
Ala204Gly + Trp208Gln + Thr212Gln + Ser216Asp +   Tyr218Gln + Leu221Met + Gly223Ser
Gly206Gln + Ile209Leu + Tyr210Ala + Thr212Gly +   Tyr213Gly + Ser216Asp + Gly223Gln
Ala204Gln + Pro205Asn + Gly206Asn + Trp208Ile +   Tyr210Gln + Thr215Gln + Ala219Asp
Ala204Asn + Pro205Gln + Ile209Ala + Tyr213Leu +   Thr217Glu + Tyr218Met + Leu221Gln
Pro205Gly + Ile209Gln + Thr212Ser + Ala219Gln +   Ser220Glu + Leu221Cys + Thr224Ser

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Gly206Asn + Trp208Cys + Thr212Gly + Tyr213Gly +
    Ser216Asp + Thr217Gly + Leu221His
Ala204Gln + Pro205Ser + Tyr210His + Thr212Pro +
    Tyr218Pro + Ala219Ser + Thr224Glu
Ala204Gly + Gly206Asn + Tyr210Cys + Tyr213Thr +
    Thr215Gly + Gly223Asp + Thr224Asn
Ala204Gln + Pro205Ser + Gly206Gln + Thr212Asn +
    Tyr218Cys + Ala219His + Gly223Glu
Ala204Ser + Gly206Asn + Ile209Gly + Pro214Gly +
    Thr217Gly + Leu221Val + Thr224Pro
Pro205Gly + Ile209Gln + Pro214Asn + Tyr218Ile +
    Ala219Gly + Leu221Met + Ser222Asp
Ala204Asn + Trp208Ala + Ile209Gln + Pro214Gly +
    Thr217Glu + Tyr218Leu + Gly223Asn
Gly206Asn + Tyr210Glu + Tyr213Gly + Thr217Gln +
    Tyr218Leu + Ala219Thr + Thr224Ser
Gly206Pro + Ile209His + Tyr213Asn + Pro214Asn +
    Thr215Gln + Tyr218Ser + Leu221Thr
Pro205Gly + Gly206Ser + Trp208His + Tyr213Thr +
    Ala219Gln + Ser220Glu + Gly223Ser
Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu +
    Thr212Asn + Tyr218Met + Ser220Glu
Ala204His + Gly206Asn + Tyr210Ser + Pro214Ser +
    Thr217Gln + Tyr218His + Ser222Asp
Gly206Gln + Tyr213Asn + Thr215Gly + Ser216Glu +
    Thr217Gly + Tyr218His + Gly223Asn
Gly206Gln + Ser207Glu + Tyr210Gly + Thr212Pro +
    Tyr213Met + Gly223Pro + Thr224Pro
Ala204Asn + Trp208Thr + Ile209Gln + Tyr213Pro +
    Thr217Ser + Leu221Pro + Ser222Asp
Ala204Ser + Gly206Gln + Trp208Asn + Thr212Ser +
    Ala219Gln + Ser220Glu + Leu221Asn
Pro205Gly + Gly206Gln + Ile209Asn + Tyr213Val +
    Thr215Gln + Thr217Ser + Ala219Thr
Ala204Ser + Pro205Gln + Ile209Asn + Thr212Asn +
    Thr217Glu + Gly223Pro + Thr224Ser
Thr212Gly + Tyr213Ile + Pro214Gly + Tyr218Gly +
    Ala219Pro + Leu221Asn + Thr224Glu
Ala204Asn + Tyr210Asp + Thr212Pro + Tyr213Asn +
    Thr217Asn + Tyr218Cys + Leu221His
Ala204Gln + Ile209Cys + Tyr210Gln + Thr212Gly +
    Tyr213Gln + Gly223Pro + Thr224Asp
Pro205Gln + Gly206Gln + Trp208Thr + Tyr210Leu +
    Thr215Gly + Thr217Gly + Tyr218Leu
Tyr210Ala + Tyr213Val + Pro214Gln + Ala219Ser +
    Leu221Cys + Ser222Glu + Thr224Asn
Ala204Asn + Gly206Pro + Ser207Asp + Trp208His +
    Ile209His + Pro214Gln + Leu221Gln
Ala204Asn + Gly206Asn + Trp208Ile + Ile209Asn +
    Ser216Glu + Ala219Pro + Gly223Asn
Ala204Ser + Gly206Ser + Ile209Ala + Thr212Ser +
    Pro214Gln + Tyr218Asp + Leu221His
Gly206Asn + Ile209Pro + Tyr213Cys + Thr215Gln +
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Ser216Glu + Thr217Pro + Ala219His
Ala204Gln + Trp208His + Tyr210Gly + Thr212Gln +
    Tyr218Met + Ala219Asn + Ser220Glu
Ala204Pro + Ser207Asp + Tyr213Val + Pro214Gly +
    Thr215Gly + Tyr218Ala + Leu221Gln
Pro205Asn + Gly206Pro + Trp208Leu + Tyr210Pro +
    Pro214Asn + Thr217Gln + Tyr218Glu
Pro205Asn + Gly206Gln + Tyr213Val + Ala219Gln +
    Ser220Asp + Gly223Ser + Thr224Asn
Gly206Pro + Ile209Cys + Tyr210Ser + Thr212Asn +
    Tyr213Cys + Pro214Gln + Tyr218Cys
Ala204His + Trp208Asn + Thr212Pro + Pro214Gln +
    Thr215Asn + Thr217Gly + Thr224Asp
Pro205Ser + Gly206Ser + Tyr210Ser + Thr215Pro +
    Ala219Asn + Leu221Met + Ser222Asp
Ala204Ser + Gly206Gln + Trp208Pro + Thr212Asn +
    Thr215Glu + Tyr218Ile + Gly223Asn
Ala204Ser + Pro205Asn + Trp208Tyr + Thr215Gly +
    Tyr218Pro + Ser220Glu + Thr224Gly
Ala204Asn + Tyr210His + Pro214Asn + Tyr218Pro +
    Ala219Gln + Ser220Glu + Leu221Ile
Ala204Gly + Gly206Asn + Ser207Asp + Thr212Asn +
    Tyr213Leu + Thr215Pro + Gly223Pro
Ala204Asn + Tyr210Met + Thr212Gln + Pro214Gly +
    Ala219Gly + Ser222Asp + Gly223Gln
Tyr210Pro + Thr212Gly + Tyr213Leu + Thr215Gly +
    Thr217Gly + Ala219Glu + Ser220Asp
Gly206Ser + Trp208Asn + Ile209Pro + Ala219Asp +
    Ser220Asp + Gly223Ser + Thr224Gly
Pro205Ser + Tyr210Cys + Tyr213Thr + Pro214Asp +
    Thr215Asp + Tyr218Val + Leu221Ser
Trp208His + Ile209Val + Thr212Pro + Tyr213Leu +
    Thr217Ser + Ser222Glu + Gly223Glu
Pro205Gly + Gly206Gln + Tyr210Asn + Tyr213Gly +
    Thr217Gly + Ser222Glu + Gly223Glu
Ala204Thr + Thr212Gln + Tyr213Gln + Thr217Ser +
    Ser222Asp + Gly223Asp + Thr224Pro
Pro205Gly + Ile209Cys + Tyr213Val + Pro214Gln +
    Thr215Pro + Ser222Glu + Gly223Asp
Trp208Ser + Ile209Ser + Ser216Glu + Thr217Glu +
    Tyr218Gly + Ala219His + Gly223Pro
Gly206Pro + Tyr210Gln + Thr212Pro + Tyr213Leu +
    Ser216Glu + Thr217Asp + Leu221Pro
Trp208Met + Ile209Gln + Tyr210Met + Tyr218Gln +
    Ser220Glu + Leu221Glu + Thr224Ser
Ile209Gln + Thr212Ser + Tyr213Val + Thr215Asp +
    Ser216Glu + Tyr218Met + Ala219Pro
Tyr213Pro + Pro214Gln + Thr215Asp + Ser216Glu +
    Thr217Asp + Tyr218Met + Leu221Ala
Ala204His + Pro205Gly + Ser207Glu + Trp208His +
    Tyr210His + Tyr213Cys + Ser222Asp
Gly206Pro + Ser207Asp + Trp208Ser + Pro214Ser +
    Leu221Asn + Ser222Asp + Gly223Pro
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Ser207Glu + Trp208Tyr + Ile209Gly + Tyr213Thr +
    Pro214Ser + Tyr218Met + Ser222Glu
Pro205Ser + Ser207Asp + Trp208Ser + Thr212Gly +
    Tyr213Asn + Tyr218Gln + Ser222Asp
Ala204Thr + Gly206Asn + Trp208Thr + Thr212Gln +
    Pro214Glu + Thr215Asp + Ser216Asp
Ala204Thr + Gly206Asn + Ser207Glu + Tyr210Ile +
    Leu221Thr + Ser222Glu + Gly223Glu
Ala204His + Ser207Glu + Thr215Gln + Thr217Gln +
    Tyr218Asn + Ser222Glu + Gly223Glu
Ala204Gln + Trp208Asp + Tyr210Thr + Thr212Ser +
    Thr217Asn + Ser222Asp + Gly223Asn
Ile209Thr + Ser216Asp + Thr217Asp + Tyr218Asp +
    Leu221Ser + Gly223Ser + Thr224Gly
Ala204Asn + Gly206Gln + Ser207Glu + Trp208His +
    Thr212Ser + Leu221Asp + Ser222Asp
Gly206Ser + Ser207Asp + Tyr210Val + Thr212Gln +
    Tyr218Ile + Leu221Asp + Ser222Asp
Ala204Ser + Gly206Pro + Pro214Gly + Thr215Asp +
    Thr217Asp + Tyr218Pro + Gly223Pro
Gly206Gln + Ile209His + Pro214Asp + Thr215Pro +
    Ser216Asp + Leu221Gly + Thr224Ser
Gly206Asn + Ser207Glu + Trp208Asp + Ile209Gly +
    Tyr213Gly + Ser220Glu + Ser222Glu
Pro205Ser + Ser207Asp + Ile209Asn + Tyr213Met +
    Thr217Gln + Ala219His + Gly223Glu
Pro205Gly + Gly206Gln + Ser207Glu + Ala219Pro +
    Ser220Asp + Leu221Glu + Thr224Ser
Trp208Met + Tyr210Thr + Thr212Asn + Ala219Gln +
    Leu221Asp + Gly223Asp + Thr224Gln
Pro205Ser + Ser207Glu + Tyr213His + Thr217Pro +
    Ala219Ser + Ser220Glu + Ser222Glu
Ser207Asp + Tyr210Met + Pro214Ser + Tyr218Asn +
    Ser220Glu + Leu221Cys + Ser222Glu
Ala204Pro + Ser207Glu + Pro214Gln + Ser220Asp +
    Leu221His + Ser222Asp + Thr224Ser
Ser207Glu + Ile209Met + Tyr210Gly + Tyr213Leu +
    Thr215Gln + Ser220Glu + Ser222Asp
Gly206Pro + Ser207Asp + Ile209Ser + Tyr210His +
    Thr217Gly + Ser222Glu + Thr224Asp
Pro205Asn + Tyr210Cys + Tyr213Gly + Thr217Asn +
    Tyr218Met + Ser220Glu + Ser222Asp
Ile209Gly + Thr212Pro + Thr215Asn + Thr217Gly +
    Ala219His + Ser220Glu + Ser222Glu
Ala204Gln + Gly206Gln + Thr212Pro + Ser220Glu +
    Leu221Val + Ser222Asp + Thr224Gln
Ala204Gln + Pro205Ser + Gly206Ser + Trp208Gln +
    Thr215Pro + Ser220Glu + Ser222Glu
Ser207Glu + Trp208Leu + Tyr210Ile + Thr212Gly +
    Tyr218Cys + Leu221Asp + Gly223Asn
Pro205Gly + Gly206Pro + Ser207Asp + Ile209Met +
    Leu221Asp + Ser222Glu + Thr224Asp
Pro205Gly + Thr215Asn + Thr217Pro + Tyr218Val +

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Leu221Thr + Ser222Glu + Thr224Asp
Trp208Phe + Tyr213Met + Thr215Asn + Tyr218Asp +
    Ala219Pro + Ser220Glu + Thr224Gly
Ala204Pro + Pro205Gly + Trp208Leu + Thr217Gln +
    Leu221Asp + Ser222Glu + Thr224Asp
Ile209Ala + Thr212Gln + Thr217Gln + Tyr218Thr +
    Ser220Glu + Leu221Asp + Gly223Glu
Ala204Asn + Ile209Cys + Tyr213Cys + Tyr218Ala +
    Ala219Glu + Leu221Asp + Ser222Glu
Gly206Ser + Ser207Glu + Trp208His + Tyr210Asp +
    Tyr218Val + Ala219Thr + Ser222Asp
Pro205Gly + Ser207Asp + Trp208Thr + Thr212Ser +
    Thr217Gly + Ser220Glu + Gly223Ser
Ser207Glu + Trp208Asn + Tyr210Pro + Pro214Asn +
    Tyr218Ser + Ser220Asp + Gly223Ser
Ala204Gln + Ser207Asp + Trp208Gly + Ile209Ala +
    Thr212Asn + Ser220Glu + Gly223Pro
Pro205Gln + Ser207Asp + Ile209Gln + Pro214Asn +
    Thr215Gln + Ser220Asp + Thr224Gly
Pro205Asn + Ser207Glu + Ile209Val + Thr212Gly +
    Tyr213Pro + Ala219Asn + Thr224Glu
Ala204Gly + Ser207Glu + Ile209Ala + Thr212Pro +
    Tyr213Cys + Ala219Glu + Ser220Asp
Pro205Ser + Trp208Glu + Thr212Asn + Tyr218Glu +
    Ala219His + Leu221Glu + Ser222Glu
Pro205Ser + Ile209Pro + Tyr210Asp + Thr212Ser +
    Tyr213Ile + Ala219Pro + Ser222Glu
Gly206Ser + Trp208Thr + Tyr210Ala + Pro214Asp +
    Tyr218Met + Ala219Glu + Ser220Asp
Ile209Leu + Pro214Glu + Thr215Pro + Ala219Glu +
    Ser220Glu + Gly223Pro + Thr224Gly
Trp208Tyr + Thr212Asn + Thr217Glu + Ala219Gly +
    Ser220Asp + Leu221Glu + Ser222Asp
Ala204Thr + Tyr210Pro + Tyr213Asn + Pro214Glu +
    Tyr218Asp + Ser220Asp + Thr224Gly
Gly206Pro + Thr212Pro + Pro214Gly + Thr215Pro +
    Ser220Asp + Leu221Asp + Thr224Glu
Thr212Asn + Pro214Gln + Tyr218Met + Ala219Asp +
    Ser222Glu + Gly223Asp + Thr224Glu
Trp208Gly + Tyr210Pro + Thr212Gly + Ser220Glu +
    Leu221Met + Gly223Asp + Thr224Glu
Gly206Gln + Tyr213Asn + Pro214Asn + Thr217Asp +
    Ala219His + Ser220Glu + Leu221Asp
Pro205Gln + Ile209Pro + Tyr210Asn + Tyr218Thr +
    Ala219Glu + Ser220Asp + Gly223Asp
Ala204Thr + Ser207Asp + Tyr210Asp + Tyr213Met +
    Ala219Asn + Ser220Glu + Thr224Asp
Pro205Ser + Trp208Pro + Ile209Pro + Tyr213Ile +
    Ala219Asp + Ser222Asp + Gly223Asp
Ala204Asn + Thr212Pro + Thr217Glu + Tyr218Asp +
    Ser220Glu + Ser222Asp + Thr224Gln
Gly206Pro + Ser207Asp + Trp208Val + Tyr210Thr +
    Tyr213Ala + Ser220Asp + Thr224Asp
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Pro205Gly + Ser207Glu + Tyr210Ile + Pro214Ser +
    Thr217Gly + Ser220Asp + Thr224Glu
Ala204Thr + Tyr210Cys + Thr212Pro + Tyr218Ile +
    Ser220Glu + Leu221Ser + Gly223Glu
Ala204Gln + Trp208Pro + Pro214Asn + Ala219Thr +
    Ser220Glu + Gly223Asp + Thr224Gln
Pro205Gln + Tyr213Gly + Thr215Gly + Tyr218Ala +
    Ser220Asp + Gly223Glu + Thr224Pro
Pro205Gly + Gly206Ser + Thr217Pro + Ala219His +
    Ser220Asp + Leu221Cys + Gly223Asp
Pro205Ser + Tyr210Thr + Thr212Asn + Tyr213Cys +
    Pro214Ser + Ala219Asp + Ser222Asp
Trp208Asn + Thr217Glu + Tyr218Cys + Ala219Glu +
    Ser220Asp + Gly223Glu + Thr224Ser
Pro205Ser + Gly206Pro + Trp208Pro + Ile209Ala +
    Thr215Asp + Tyr218Asp + Ser220Asp
Trp208Thr + Thr212Gly + Tyr213Ile + Thr215Asp +
    Leu221Asp + Ser222Glu + Gly223Glu
Ala204Gly + Pro205Gly + Tyr210Glu + Thr217Glu +
    Tyr218Cys + Ala219Gly + Leu221Val
Ser207Asp + Thr215Pro + Thr217Asp + Tyr218Ile +
    Leu221Ala + Ser222Glu + Gly223Asp
Gly206Pro + Trp208Glu + Tyr210Cys + Pro214Gln +
    Thr217Asp + Ser220Asp + Gly223Gln
Trp208Thr + Tyr213Pro + Pro214Ser + Ser216Asp +
    Tyr218Met + Ala219Asp + Thr224Pro
Ala204Asn + Trp208Ala + Tyr213Asn + Ser216Asp +
    Ala219Glu + Leu221Cys + Thr224Gly
Gly206Ser + Tyr210Ser + Pro214Gly + Ser216Asp +
    Tyr218Cys + Ala219Glu + Leu221Pro
Ala204Thr + Tyr210Gln + Thr212Gln + Tyr213Ala +
    Pro214Gln + Thr217Asp + Ser220Glu
Pro205Gln + Gly206Ser + Ser207Glu + Tyr210Ser +
    Ser216Asp + Thr217Glu + Tyr218Glu
Tyr210Met + Tyr213Met + Ser216Asp + Ala219Glu +
    Ser220Glu + Leu221Cys + Ser222Glu
Ala204Gln + Ile209His + Ser216Glu + Thr217Ser +
    Ala219Glu + Ser220Asp + Ser222Glu
Ile209Ser + Tyr210Leu + Tyr213Leu + Pro214Asp +
    Thr217Asp + Ser220Glu + Leu221Glu
Ala204His + Pro205Gln + Pro214Asn + Tyr218Gln +
    Ala219Asp + Leu221Glu + Thr224Asp
Ala204Gly + Ser207Glu + Pro214Glu + Tyr218Pro +
    Ser220Glu + Ser222Asp + Thr224Asn
Ala204Pro + Pro205Gly + Trp208Glu + Ile209Ser +
    Tyr210Asp + Ala219Thr + Thr224Glu
Ala204Gly + Ser207Glu + Trp208Glu + Tyr210Asp +
    Ser216Glu + Thr217Gln + Thr224Gly
Trp208Ala + Tyr210Cys + Thr212Ser + Thr217Glu +
    Ala219Gln + Ser220Asp + Ser222Glu
Gly206Asn + Ser207Glu + Trp208Glu + Thr212Ser +
    Ser216Glu + Leu221Met + Gly223Glu
Ala204Thr + Ser207Asp + Ile209Cys + Thr215Asp +

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Tyr218Ile + Ser220Asp + Leu221Asp
Ser207Asp + Thr212Pro + Pro214Gln + Ser216Glu +
    Tyr218Val + Ser220Asp + Ser222Asp
Ile209Pro + Thr212Gly + Tyr213Ser + Pro214Glu +
    Thr215Glu + Ala219Gln + Ser220Glu
Pro205Asn + Ser207Glu + Ile209Met + Pro214Ser +
    Ser216Asp + Ala219Glu + Leu221Asp
Ala204Thr + Ser207Glu + Tyr210Leu + Ser216Glu +
    Ala219Asp + Leu221Asp + Gly223Asn
Gly206Asn + Ser207Glu + Pro214Gly + Thr215Glu +
    Thr217Glu + Tyr218Thr + Ala219Glu
Gly206Gln + Ser207Glu + Tyr210Ala + Thr215Asn +
    Thr217Asp + Ser220Asp + Leu221Ala
Ser207Glu + Trp208Leu + Tyr213Val + Pro214Asp +
    Ala219Glu + Ser222Glu + Thr224Pro
Ala204Thr + Ser207Asp + Trp208Leu + Tyr210Asp +
    Thr212Pro + Thr215Asp + Leu221Glu
Pro205Gln + Ser207Asp + Ile209Ala + Thr217Asn +
    Tyr218Glu + Ala219Asp + Gly223Asp
Ser207Glu + Ile209Thr + Tyr213Gly + Ser216Asp +
    Ala219Asp + Ser222Glu + Gly223Pro
Gly206Ser + Ser207Asp + Ile209Ala + Thr212Gln +
    Thr217Glu + Ala219Glu + Leu221Asn
Pro205Asn + Tyr210Glu + Thr217Asp + Tyr218Ala +
    Leu221Ile + Ser222Glu + Thr224Pro
Ser216Asp + Thr217Asp + Tyr218Cys + Ala219His +
    Leu221Glu + Ser222Glu + Gly223Asn
Ala204Gln + Trp208Asp + Pro214Gln + Ser216Glu +
    Thr217Gln + Ser220Asp + Gly223Asp
Pro205Gln + Tyr210Leu + Tyr213Thr + Ser216Glu +
    Tyr218Glu + Ser220Glu + Gly223Glu
Pro214Gly + Thr217Asp + Tyr218Gly + Ala219Glu +
    Leu221Asn + Ser222Glu + Thr224Glu
Gly206Pro + Ser207Asp + Trp208Asp + Thr212Gln +
    Thr217Asp + Gly223Ser + Thr224Glu
Ala204Pro + Pro205Ser + Gly206Pro + Ile209Val +
    Tyr213Pro + Thr215Asp + Ala219Glu
Ala204Pro + Gly206Pro + Tyr210Val + Pro214Glu +
    Ser220Glu + Ser222Glu + Thr224Pro
Ala204Gly + Gly206Pro + Thr212Gln + Pro214Glu +
    Ser220Glu + Leu221Val + Ser222Asp
Ala204Asn + Gly206Gln + Ile209Ser + Tyr210Ala +
    Pro214Glu + Ser220Glu + Ser222Asp
Pro205Gln + Trp208Gln + Pro214Glu + Thr217Pro +
    Leu221Met + Ser222Asp + Gly223Glu
Ala204Thr + Pro205Asn + Pro214Asp + Thr215Pro +
    Leu221Cys + Ser222Asp + Gly223Asp
Ile209Pro + Tyr210Pro + Thr212Pro + Thr217Asp +
    Leu221Asn + Ser222Asp + Gly223Asp
Gly206Gln + Trp208Thr + Ile209Ser + Tyr213Met +
    Thr215Glu + Ser222Asp + Gly223Glu
Pro205Asn + Trp208Ser + Thr212Gly + Tyr213Asn +
    Thr215Asp + Ser222Asp + Gly223Glu
```

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```
    Tyr210Gly + Thr212Pro + Pro214Gly + Ser216Asp +
        Ala219His + Leu221Glu + Ser222Asp
    Pro205Gly + Gly206Pro + Ile209His + Thr215Asp +
        Ala219Gly + Leu221Glu + Ser222Glu
 5  Pro205Gln + Tyr210Gly + Thr212Asn + Ser216Asp +
        Ala219Pro + Ser220Glu + Leu221Asp
    Gly206Ser + Trp208Glu + Tyr210Gln + Thr215Gly +
        Ser216Glu + Thr217Glu + Leu221Pro
    Pro205Gln + Gly206Gln + Ser216Glu + Thr217Asp +
10      Leu221Ala + Ser222Glu + Thr224Pro
    Gly206Ser + Ser207Glu + Trp208Asp + Ile209Leu +
        Thr215Asp + Ser216Glu + Tyr218Gln
    Tyr210His + Thr212Gln + Thr215Asp + Ser216Glu +
        Leu221Glu + Gly223Ser + Thr224Pro
15  Ala204Ser + Pro205Ser + Gly206Asn + Thr215Asp +
        Ser216Glu + Tyr218Val + Gly223Asp
    Ala204Gln + Gly206Asn + Ser207Glu + Trp208Pro +
        Pro214Asp + Thr215Asp + Ser222Asp
    Ser207Asp + Tyr210Gln + Thr212Gln + Thr215Asp +
20      Ser216Asp + Leu221Gly + Ser222Asp
    Ile209Met + Tyr210Leu + Thr217Glu + Tyr218Thr +
        Ser220Glu + Gly223Glu + Thr224Asn
    Pro205Ser + Ser207Glu + Trp208His + Thr215Pro +
        Thr217Asp + Leu221Asn + Ser222Glu
25  Ala204Thr + Ser207Glu + Thr212Gly + Tyr213Leu +
        Pro214Asn + Ser216Glu + Ser222Glu
    Ala204Pro + Ser207Asp + Tyr210Leu + Thr212Gln +
        Tyr213Thr + Thr215Glu + Ser222Glu
```

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TABLE 29
Multi-loop Double Mutation Variants
Leu104Gly + Tyr210Pro
Asn 66Gln + Thr217Glu
Gly 67Ser + Gly 69Ser
Val103Gln + Gly223Asp
Trp112Cys + Thr217Gly
Ala164Glu + Trp208Gln
Leu134Ile + Trp208Gln
Ala219Gly + Ser220Glu
Asn 66Asp + Leu104Gly
Ala168Thr + Ser216Glu
Leu104Ala + Ser109Glu
Thr111Gln + Val115Ala
Ser109Asp + Tyr210Pro
Gly 69Ser + Ser191Asp
Val103Asp + Gly206Gln
Gly 67Glu + Pro214Asn
Trp208Ile + Thr215Asp
Ser107Glu + Thr113Gly
Gly108Ser + Leu221Glu
Ser 70Glu + Pro169Asn

```
Gly165Gln  +  Leu221Asp
Gly108Glu  +  Ala164Gly
Leu104His  +  Thr167Pro
Ala164His  +  Ser220Glu
Thr195Gln  +  Ser222Asp
Trp208His  +  Ala219Pro
Gly 69Glu  +  Asn106Gln
Trp112Cys  +  Thr113Pro
Gly 67Gln  +  Trp208Asp
Ala114Glu  +  Leu134Ile
Ser109Glu  +  Gly136Ser
Gly110Ser  +  Ser207Glu
Val103Ala  +  Thr212Ser
Ala114Glu  +  Thr167Pro
Thr195Glu  +  Ala204Thr
Gly 67Ser  +  Thr217Glu
Trp208Tyr  +  Ser220Glu
Ala168His  +  Thr217Asp
Ala168Glu  +  Tyr213Asn
Gly110Pro  +  Ser220Asp
Tyr171Asn  +  Leu221Met
Thr 73Asp  +  Leu134Ser
Thr137Asn  +  Thr217Pro
Ser 70Asp  +  Ala168Thr
Thr167Glu  +  Tyr210Pro
Thr195Glu  +  Tyr210Gly
Tyr210Gln  +  Thr217Glu
Gly 67Glu  +  Val115Cys
Ser 70Glu  +  Ala219Thr
Gly136Pro  +  Leu221Ala
Gly 67Ser  +  Thr195Ser
Phe193His  +  Ser222Asp
Asn 68Glu  +  Tyr210Ser
Gly165Asp  +  Pro169Ser
Trp208Pro  +  Thr215Gly
Thr 73Gly  +  Leu104Gly
Thr113Asn  +  Ser216Glu
Gly 72Gln  +  Gly139Asp
Tyr210Ile  +  Leu221Gly
Thr 73Pro  +  Tyr171Asp
Leu104Gln  +  Thr137Gly
Gly 69Pro  +  Tyr210Ala
Thr217Gly  +  Gly223Glu
Thr195Asp  +  Tyr213Ser
Gly 72Asn  +  Ala219Asn
Val138Pro  +  Tyr210Ala
Tyr218Met  +  Gly223Asn
Ser220Glu  +  Leu221Asn
Trp112Ala  +  Gly139Asn
Gly135Glu  +  Leu221Gly
Thr113Asn  +  Thr217Ser
Thr195Asn  +  Thr217Asp
Tyr210Val  +  Ser220Glu
```

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```
Val138Gly  +  Ala219Asp
Asn166Glu  +  Leu221His
Gly165Gln  +  Leu221His
Thr113Gln  +  Ser192Asp
Asn 66Asp  +  Thr 73Ser
Thr167Gln  +  Thr217Gly
Val115Pro  +  Gly135Ser
Ala219Gln  +  Gly223Asp
Trp208His  +  Thr217Asn
Trp208Leu  +  Tyr210Val
Gly108Asn  +  Ser191Glu
Ser107Glu  +  Tyr210Ala
Asn 68Gln  +  Ser220Glu
Trp112Cys  +  Leu221Asn
Gly108Gln  +  Ser109Asp
Val103Met  +  Ala168His
Ala164Pro  +  Ser192Glu
Pro214Ser  +  Ser220Glu
Thr215Asn  +  Thr217Gln
Ser220Glu  +  Leu221Pro
Gly136Ser  +  Trp208Phe
Thr195Asp  +  Tyr210Gln
Leu104Gln  +  Ala219Asp
Gly 69Pro  +  Leu221Pro
Gly 69Asn  +  Ala204Gly
Gly135Glu  +  Ala219Asn
Gly136Asn  +  Ser220Glu
Gly136Ser  +  Trp208Val
Gly139Asp  +  Asn140Gln
Trp208Cys  +  Tyr210Asn
Gly165Ser  +  Ser220Asp
Gly110Ser  +  Trp208Thr
Ala114Asp  +  Gly162Asn
Ser192Asp  +  Leu221Cys
Val103Ser  +  Gly135Glu
Asn140Glu  +  Trp208Ala
Thr113Asp  +  Leu221Ser
Ser109Asp  +  Gly110Ser
Ser109Glu  +  Thr113Gln
Thr 73Gly  +  Gly136Asp
Ala164His  +  Leu221Asn
Gly 67Pro  +  Val103His
Ala114Asn  +  Thr212Gln
Asn140Asp  +  Gly223Pro
Gly139Gln  +  Pro214Asn
Asp105Glu  +  Thr195Gly
Gly135Pro  +  Ser207Glu
Leu104Glu  +  Ala114Pro
Asn 66Glu  +  Gly 69Pro
Val115Cys  +  Ser207Glu
Asn 68Glu  +  Leu221Ala
Asn 66Ser  +  Gly 69Asp
Ser109Glu  +  Phe193Pro
```

```
Trp208Glu + Thr212Pro
Gly136Gln + Trp208Cys
Ser 70Asp + Leu221His
Ser 70Glu + Phe193Ser
Thr217Asn + Ser222Glu
Tyr213Thr + Tyr218Gly
Trp208Ser + Leu221Cys
Asn163Asp + Leu221Ser
Asn 66Gln + Gly223Pro
Gly139Asn + Ser220Asp
Thr 73Ser + Pro214Asp
Ser 70Asp + Leu221Ile
Gly165Gln + Ser220Asp
Asn166Asp + Thr217Asn
Asp105Glu + Thr111Pro
Asn140Asp + Trp208Phe
Asn166Glu + Pro214Asn
Trp208Ala + Thr217Ser
Gly108Ser + Gly110Glu
Val138Asn + Asn170Asp
Gly110Glu + Thr224Gln
Trp208Met + Ser220Asp
Asn 68Glu + Val115Ser
Asn170Glu + Tyr210Gly
Gly110Asn + Ser192Glu
Trp208Gln + Ser220Glu
Asn140Gln + Pro214Gln
Thr217Ser + Ser220Glu
Ser141Glu + Ala168Thr
Gly162Glu + Tyr210His
Leu104Gly + Thr224Asp
Trp112Pro + Ser191Asp
Trp208Thr + Tyr210Glu
Gly108Gln + Thr111Asp
Leu134Val + Thr217Pro
Asn170Asp + Tyr210His
Val115Ser + Ser220Glu
Gly 69Pro + Ser 70Asp
Thr 73Gly + Tyr210Glu
Gly 67Asp + Gly108Gln
Gly 69Ser + Gly110Asp
Tyr210Thr + Ser220Glu
Trp208Phe + Leu221Met
Gly136Gln + Leu221Asp
Thr113Ser + Trp208Val
Tyr171Leu + Tyr210Thr
Leu134Asn + Thr167Gln
Asn140Ser + Tyr210Asp
Asn 66Gln + Gly 67Glu
Leu134Cys + Pro214Asn
Gly136Ser + Leu221Ala
Thr111Pro + Trp208Val
Thr113Asp + Tyr171Gly
```

```
Trp208Ile  +  Ser220Glu
Pro214Ser  +  Thr224Glu
Gly165Pro  +  Ser220Glu
Thr137Gln  +  Tyr171Glu
Trp208Glu  +  Pro214Gly
Ser216Asp  +  Leu221Met
Gly136Ser  +  Thr217Gln
Ser141Glu  +  Tyr210Leu
Trp208Thr  +  Gly223Ser
Asn 66Ser  +  Asn170Gln
Asn 66Gln  +  Asn170Glu
Pro169Glu  +  Tyr210Thr
Tyr171Leu  +  Leu221Asp
Gly139Glu  +  Leu221Pro
Thr 73Pro  +  Leu134Gly
Tyr210Glu  +  Leu221Ala
Gly139Ser  +  Tyr210Gly
Asn163Asp  +  Ala164Ser
Asn163Gln  +  Thr217Asp
Asn140Asp  +  Tyr218Ser
Gly108Ser  +  Ala164Gln
Val138Ser  +  Trp208Leu
Thr217Ser  +  Thr224Asp
Ser109Asp  +  Tyr218Ala
Asn 66Glu  +  Phe193Val
Asn140Asp  +  Ala204Gly
Val138Ser  +  Ser220Asp
Gly108Asn  +  Tyr210Thr
Gly139Asn  +  Tyr171Pro
Thr113Pro  +  Trp208Thr
Ser141Glu  +  Leu221Gly
Asn106Glu  +  Thr167Gly
Trp112Ser  +  Ala219Ser
Asp105Glu  +  Asn166Gln
Ala114Pro  +  Tyr171Asn
Thr217Gln  +  Ser220Asp
Thr113Gly  +  Tyr210Asp
Tyr171Cys  +  Tyr210Glu
Asn166Asp  +  Leu221Val
Ala114His  +  Ser191Asp
Gly 67Glu  +  Thr217Asn
Asn 66Glu  +  Thr215Gln
Thr111Asn  +  Ser220Glu
Trp208Gln  +  Leu221Ser
Gly 69Gln  +  Ala114Thr
Asn163Asp  +  Leu221Ala
Pro214Gly  +  Leu221Ile
Thr217Gly  +  Ser220Asp
Ala219Thr  +  Ser220Asp
Tyr210Cys  +  Ser220Glu
Val138Asp  +  Gly223Pro
Thr195Asn  +  Leu221Gly
Leu104Thr  +  Thr215Ser
```

```
Gly 69Gln + Leu134Thr
Gly165Pro + Leu221Gly
Thr167Ser + Thr217Glu
Thr212Pro + Thr217Glu
Asn166Ser + Tyr218Asp
Gly108Glu + Tyr171Gln
Gly 67Asn + Tyr210Ile
Val115Cys + Pro214Gly
Thr137Asp + Tyr210Val
Leu104Met + Trp208Gly
Leu134Ala + Ser192Asp
Trp112Gln + Ala219Gln
Gly108Glu + Val115His
Thr113Asn + Gly139Pro
Trp208Gln + Gly223Asn
Ser207Glu + Trp208Gln
Asn 66Ser + Tyr210Asn
Ser207Glu + Tyr210Pro
Val103Ala + Thr215Glu
Val103Asn + Tyr210Ala
Asn 66Ser + Trp208Phe
Thr215Asn + Ala219Thr
Gly136Asn + Gly139Asn
Tyr171Thr + Ser216Glu
Leu104Glu + Tyr210Asn
Ser109Glu + Thr217Asn
Leu134Ile + Asn166Ser
Tyr171Gly + Thr217Gln
Val115Cys + Thr224Asp
Pro169Ser + Thr215Asp
Gly 72Gln + Gly135Asn
Tyr210Thr + Ser220Asp
Asn 68Glu + Thr113Ser
Asn106Gln + Thr113Asp
Ser109Glu + Trp208Asn
Leu134Gln + Thr137Glu
Ala114Thr + Asn170Glu
Ala219Pro + Ser220Glu
Tyr171Val + Trp208Pro
Gly139Ser + Tyr213Ser
Ser107Asp + Thr113Asn
Thr167Gly + Leu221Met
Tyr171Gly + Ser207Glu
Gly 67Ser + Thr217Ser
Val115Gly + Thr217Ser
Asn 66Ser + Trp208Ile
Ser191Glu + Trp208Cys
Thr137Asp + Trp208Ser
Leu221Gly + Ser222Glu
Ser216Glu + Leu221Thr
Gly162Pro + Ser207Glu
Thr217Asp + Ala219Thr
Leu104Cys + Ser207Glu
```

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```
Gly 69Gln  +  Ser220Glu
Gly 69Gln  +  Val138Asp
Pro169Gly  +  Tyr210Leu
Thr 73Asp  +  Gly110Ser
Thr215Gly  +  Thr217Ser
Gly 69Ser  +  Gly 72Asn
Ser191Glu  +  Thr217Asn
Gly135Ser  +  Tyr171Ser
Thr111Asn  +  Phe193Leu
Trp112Leu  +  Ser207Asp
Leu104Glu  +  Trp208Ala
Leu221Ile  +  Thr224Asp
Gly136Glu  +  Gly165Asn
Tyr210Glu  +  Thr217Pro
Trp112Phe  +  Pro214Asp
Gly110Gln  +  Trp112Met
Asn 68Ser  +  Trp208Asp
Gly 69Ser  +  Leu104Cys
Thr137Glu  +  Thr215Pro
Asn166Gln  +  Thr217Glu
Tyr210Thr  +  Thr212Ser
Leu134His  +  Trp208Asp
Ser141Glu  +  Tyr210Val
Ser191Glu  +  Tyr210Leu
Gly139Pro  +  Ser207Asp
Gly162Ser  +  Tyr171Met
Thr 73Gln  +  Val103Ala
Tyr171Val  +  Phe193Asp
Leu221Thr  +  Ser222Glu
Gly 69Asp  +  Ala114Gln
Gly108Glu  +  Trp208Met
Gly 72Ser  +  Tyr210Gln
Thr137Asn  +  Trp208Asp
Asn140Asp  +  Phe193Thr
Ser192Glu  +  Trp208Ala
Asn170Glu  +  Pro205Ser
Tyr210Ala  +  Tyr213Ser
Tyr171Gln  +  Tyr218Ile
Asn 66Asp  +  Leu221Cys
Asn163Gln  +  Gly223Asn
Val103Thr  +  Leu134Ser
Thr167Ser  +  Asn170Asp
Ser 70Glu  +  Gly162Ser
Tyr171Gln  +  Ser192Glu
Tyr210Val  +  Gly223Ser
Thr137Glu  +  Leu221Pro
Gly 69Asn  +  Thr217Asp
Pro205Ser  +  Tyr218Glu
Tyr210Met  +  Leu221Asn
Thr215Asp  +  Thr217Pro
Leu134Val  +  Gly136Glu
Thr 73Pro  +  Leu221Glu
Tyr213Val  +  Ser220Glu
```

```
Thr 73Ser  +  Ser107Glu
Trp208Ser  +  Leu221Pro
Thr113Asp  +  Leu221His
Ala114Thr  +  Thr217Asp
Ala168Ser  +  Tyr218Glu
Trp208Asp  +  Leu221Asn
Thr137Asp  +  Trp208Ile
Gly135Gln  +  Ser191Glu
Val103Glu  +  Trp208Gly
Asn140Asp  +  Leu221His
Ser192Asp  +  Tyr210Cys
Gly108Asn  +  Tyr210His
Asp105Glu  +  Trp208Asn
Val103Pro  +  Tyr210Pro
Thr 73Gln  +  Trp112Tyr
Leu134Cys  +  Asn170Ser
Asn166Glu  +  Tyr210Cys
Asn106Ser  +  Leu221Met
Ser109Glu  +  Leu221Cys
Ser107Asp  +  Leu221Ala
Ala114Ser  +  Leu221Asn
Gly162Asn  +  Leu221Val
```

TABLE 33

Multi-loop Triple Mutation Variants

```
Leu104Gly  +  Tyr210Pro  +  Thr217Glu
Asn 66Gln  +  Gly 67Ser  +  Gly 69Ser
Val103Gln  +  Trp112Cys  +  Gly223Asp
Ala164Glu  +  Trp208Gln  +  Thr217Gly
Leu134Ile  +  Trp208Gln  +  Ser220Glu
Asn 66Asp  +  Leu104Gly  +  Ala219Gly
Leu104Ala  +  Thr111Gln  +  Val115Ala
Gly 69Ser  +  Ser109Asp  +  Tyr210Pro
Gly139Asn  +  Thr167Asn  +  Thr217Ser
Gly108Glu  +  Ala164Gly  +  Gly165Gln
Leu104His  +  Thr167Pro  +  Ser220Asp
Gly 69Glu  +  Asn106Gln  +  Trp208His
Trp112Cys  +  Thr113Pro  +  Trp208Asp
Gly110Ser  +  Ser207Glu  +  Thr212Ser
Ala168His  +  Trp208Tyr  +  Ser220Glu
Tyr171Asn  +  Thr217Asp  +  Leu221Met
Ser 70Asp  +  Thr137Asn  +  Thr217Pro
Thr167Glu  +  Ala168Thr  +  Tyr210Pro
Ser 70Glu  +  Gly136Pro  +  Leu221Ala
Gly 67Ser  +  Thr195Ser  +  Ser222Asp
Pro169Ser  +  Trp208Pro  +  Thr215Gly
Thr 73Gly  +  Leu104Gly  +  Tyr171Glu
Gly 72Gln  +  Tyr210Ile  +  Leu221Gly
Leu104Gln  +  Thr137Gly  +  Tyr210Ala
Tyr213Ser  +  Thr217Gly  +  Gly223Glu
Gly 72Asn  +  Thr195Asp  +  Ala219Asn
```

```
Thr137Asp  +  Val138Pro  +  Tyr210Ala
Ser109Glu  +  Val115Met  +  Trp208Cys
Gly139Asn  +  Ser220Glu  +  Leu221Asn
Trp112Ala  +  Gly135Glu  +  Leu221Gly
Thr113Asn  +  Thr195Asn  +  Thr217Ser
Ala164His  +  Ser220Asp  +  Leu221His
Asn 66Asp  +  Thr 73Ser  +  Thr113Gln
Gly135Ser  +  Thr167Gln  +  Thr217Gly
Tyr171Asp  +  Trp208His  +  Thr217Asn
Gly108Asn  +  Trp208Leu  +  Tyr210Val
Trp112Cys  +  Ser220Glu  +  Leu221Asn
Val103Met  +  Ala168His  +  Ser191Asp
Pro214Ser  +  Thr217Gln  +  Ser220Glu
Thr215Asn  +  Ser220Glu  +  Leu221Pro
Gly136Ser  +  Trp208Phe  +  Tyr210Gln
Gly 69Pro  +  Ala219Asp  +  Leu221Pro
Gly 69Asn  +  Ala204Gly  +  Ser220Asp
Gly136Ser  +  Gly139Asp  +  Trp208Val
Asn140Gln  +  Trp208Cys  +  Tyr210Asn
Gly110Ser  +  Gly165Ser  +  Trp208Thr
Val103Ser  +  Gly135Glu  +  Leu221Cys
Asn140Glu  +  Trp208Ala  +  Leu221Ser
Gly 67Pro  +  Val103His  +  Ala164His
Asn140Asp  +  Thr212Gln  +  Gly223Pro
Gly139Gln  +  Thr195Gly  +  Pro214Asn
Gly 69Pro  +  Leu104Glu  +  Ala114Pro
Phe193Pro  +  Trp208Glu  +  Thr212Pro
Gly136Gln  +  Trp208Cys  +  Leu221His
Ser 70Asp  +  Thr 73Gly  +  Phe193Ser
Tyr213Thr  +  Tyr218Gly  +  Gly223Asp
Thr195Glu  +  Trp208Ser  +  Leu221Cys
Asn163Asp  +  Leu221Ser  +  Gly223Pro
Asn 66Gln  +  Gly139Asn  +  Ser220Asp
Thr 73Ser  +  Pro214Asp  +  Leu221Ile
Thr111Pro  +  Asn140Asp  +  Trp208Phe
Asn166Glu  +  Pro214Asn  +  Thr217Ser
Gly108Ser  +  Gly110Glu  +  Trp208Ala
Leu134Asp  +  Gly165Asn  +  Tyr210Thr
Phe193Thr  +  Thr217Gln  +  Ser220Asp
Gly 67Pro  +  Trp112Gln  +  Ser192Asp
Tyr210Ile  +  Thr217Gln  +  Ser222Asp
Val103Ser  +  Leu104Ile  +  Ala164Asn
Ala114Asp  +  Thr167Gly  +  Thr217Asn
Thr111Asp  +  Thr195Gly  +  Tyr210Leu
Tyr210Thr  +  Ser220Glu  +  Leu221Met
Gly136Gln  +  Trp208Phe  +  Leu221Asp
Thr113Ser  +  Trp208Val  +  Tyr210Thr
Leu134Asn  +  Asn140Ser  +  Thr167Gln
Gly136Ser  +  Trp208Val  +  Leu221Ala
Gly165Pro  +  Pro214Ser  +  Ser220Glu
Gly136Ser  +  Tyr210Leu  +  Thr217Gln
Ser141Glu  +  Trp208Thr  +  Gly223Ser
Asn 66Ser  +  Thr113Pro  +  Asn170Glu
```

```
Thr 73Pro  +  Tyr210Thr  +  Ser216Glu
Thr 73Pro  +  Tyr210Glu  +  Leu221Ala
Gly139Ser  +  Ala164Ser  +  Tyr210Gly
Gly108Ser  +  Val138Ser  +  Ala164Gln
Trp208Leu  +  Thr217Ser  +  Thr224Asp
Asn 66Glu  +  Phe193Val  +  Tyr218Ala
Gly108Asn  +  Tyr171Pro  +  Tyr210Thr
Thr113Pro  +  Gly139Asn  +  Trp208Thr
Asp105Glu  +  Asn166Gln  +  Tyr171Asn
Ala114Pro  +  Thr217Gln  +  Ser220Asp
Gly 67Glu  +  Ala114His  +  Thr217Asn
Ser141Glu  +  Trp208Gln  +  Leu221Ser
Gly 69Gln  +  Ala114Thr  +  Asn170Asp
Asn163Asp  +  Ala219Thr  +  Leu221Ala
Ser 70Asp  +  Thr217Gly  +  Ala219Thr
Thr195Asn  +  Leu221Gly  +  Gly223Pro
Gly 69Gln  +  Leu104Thr  +  Leu134Thr
Gly139Asp  +  Gly165Pro  +  Leu221Gly
Gly 67Asn  +  Gly108Glu  +  Tyr210Ile
Val115Cys  +  Tyr210Val  +  Pro214Gly
Leu104Met  +  Thr137Asp  +  Trp208Gly
Trp112Gln  +  Leu134Ala  +  Ser192Asp
Gly108Glu  +  Val115His  +  Ala219Gln
Thr113Asn  +  Gly139Pro  +  Thr167Asp
Thr137Asp  +  Trp208Gln  +  Gly223Asn
Ser207Glu  +  Trp208Gln  +  Tyr210Asn
Asn 66Ser  +  Ser207Glu  +  Tyr210Pro
Val103Asn  +  Tyr210Ala  +  Thr215Glu
Asn 66Ser  +  Trp208Phe  +  Ala219Thr
Gly136Asn  +  Gly139Asn  +  Thr215Asn
Ser109Glu  +  Asn166Ser  +  Thr217Asn
Leu134Ile  +  Tyr171Gly  +  Thr217Gln
Gly 72Gln  +  Gly110Asp  +  Gly135Asn
Asn 68Glu  +  Asn106Gln  +  Thr113Ser
Ala114Thr  +  Leu134Gln  +  Thr137Glu
Ser107Asp  +  Gly139Ser  +  Tyr213Ser
Thr113Asn  +  Thr167Gly  +  Leu221Met
Gly 67Ser  +  Ser207Glu  +  Thr217Ser
Asn 66Ser  +  Trp208Ile  +  Thr217Ser
Trp208Ser  +  Leu221Gly  +  Ser222Glu
Gly162Pro  +  Ser216Glu  +  Leu221Thr
Leu104Cys  +  Asn106Gln  +  Ser207Glu
Gly 69Gln  +  Pro169Gly  +  Tyr210Leu
Thr 73Asp  +  Gly110Ser  +  Thr217Ser
Gly 69Ser  +  Gly 72Asn  +  Thr215Gly
Gly135Ser  +  Tyr171Ser  +  Ser191Glu
Thr111Asn  +  Phe193Leu  +  Thr217Glu
Trp208Ala  +  Leu221Ile  +  Thr224Asp
Trp112Phe  +  Pro214Asp  +  Thr217Pro
Asn 68Ser  +  Gly110Gln  +  Trp112Met
Gly 69Ser  +  Leu104Cys  +  Trp208Asp
Thr137Glu  +  Asn166Gln  +  Thr215Pro
Gly108Glu  +  Tyr210Thr  +  Thr212Ser
```

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```
Thr 73Gln  +  Gly162Ser  +  Tyr171Met
Tyr171Val  +  Phe193Asp  +  Leu221Thr
Gly 72Ser  +  Gly108Glu  +  Trp208Met
Thr137Asn  +  Trp208Asp  +  Tyr210Gln
Asn140Asp  +  Phe193Thr  +  Trp208Ala
Gly 69Glu  +  Tyr210Ala  +  Tyr213Ser
Asn 66Asp  +  Tyr218Ile  +  Leu221Cys
Val103Thr  +  Leu34Ser   +  Asn163Gln
Tyr171Gln  +  Ser192Glu  +  Gly223Ser
Gly 69Asn  +  Thr137Glu  +  Leu221Pro
Pro205Ser  +  Tyr218Glu  +  Leu221Asn
Tyr210Met  +  Thr215Asp  +  Thr217Pro
Thr 73Pro  +  Leu134Val  +  Gly136Glu
Thr 73Ser  +  Ser107Glu  +  Trp208Ser
Gly162Gln  +  Ser220Asp  +  Leu221Pro
Val103Glu  +  Gly135Gln  +  Trp208Gly
Asn140Asp  +  Tyr210Cys  +  Leu221His
Gly108Asn  +  Ser192Asp  +  Tyr210His
Asp105Glu  +  Trp208Asn  +  Tyr210Pro
Thr 73Gln  +  Val103Pro  +  Trp112Tyr
Leu134Cys  +  Asn170Ser  +  Tyr210Cys
Asn106Ser  +  Ser109Glu  +  Leu221Cys
Ser107Asp  +  Ala114Ser  +  Leu221Ala
Gly 69Asn  +  Gly162Asn  +  Leu221Asn
Gly 67Ser  +  Gly135Asp  +  Gly162Asn
Val115Gly  +  Leu34Ser   +  Ala164Gln
Asn 66Glu  +  Leu104Asn  +  Ala168Gly
Gly 69Ser  +  Asn166Glu  +  Tyr218Asn
Thr113Asp  +  Ala204Ser  +  Tyr218Ser
Asn166Gln  +  Trp208Thr  +  Thr217Asp
Gly108Glu  +  Thr217Ser  +  Tyr218Gly
Asn106Asp  +  Trp208Met  +  Thr217Gln
Gly 69Glu  +  Tyr171Val  +  Trp208His
Gly139Asn  +  Pro169Gly  +  Trp208Asn
Val115Ala  +  Thr215Gln  +  Leu221Glu
Thr111Pro  +  Pro169Gln  +  Ala219Glu
Thr 73Glu  +  Thr137Asn  +  Trp208His
Asn 66Asp  +  Asn106Ser  +  Leu221Thr
Gly 67Pro  +  Gly 69Ser  +  Thr224Ser
Val115His  +  Ser191Asp  +  Tyr218Val
Thr137Glu  +  Thr195Asn  +  Trp208Gly
Leu134Glu  +  Trp208Ser  +  Leu221Ser
Val103Ser  +  Trp208Glu  +  Thr217Asn
Gly139Asn  +  Phe193Pro  +  Leu221Cys
Asn106Gln  +  Ala164Pro  +  Asn166Asp
Asp105Glu  +  Val138Ser  +  Thr217Pro
Gly135Pro  +  Tyr171Thr  +  Ser222Glu
Ser216Asp  +  Thr217Asn  +  Thr224Gly
Ala114Gly  +  Asn140Gln  +  Tyr218Asp
Ser107Asp  +  Pro214Gly  +  Leu221Ser
Asp105Glu  +  Asn170Ser  +  Thr195Pro
Ser 70Glu  +  Tyr171Leu  +  Pro214Ser
Gly 67Asp  +  Leu104Thr  +  Gly223Asn
```

```
Gly108Asp  +  Ala114Asn  +  Tyr210Cys
Gly110Asn  +  Pro214Gly  +  Ser220Asp
Gly 69Asn  +  Pro169Gln  +  Ser220Glu
Gly162Glu  +  Trp208Ala  +  Thr217Asn
Trp112Tyr  +  Val115Pro  +  Ser220Glu
Trp208Gly  +  Tyr210Ile  +  Pro214Asp
Leu134Asn  +  Pro169Ser  +  Leu221Asp
Thr113Ser  +  Trp208Leu  +  Leu221Glu
Asn166Ser  +  Tyr218Ile  +  Ser220Glu
Trp112Pro  +  Phe193His  +  Pro214Asp
Leu104Glu  +  Val115Ser  +  Ala164Ser
Gly 69Pro  +  Pro169Asp  +  Leu221His
Gly108Gln  +  Val138Ser  +  Ser220Glu
Gly110Asn  +  Thr137Gln  +  Ser220Glu
Ala164Thr  +  Asn166Ser  +  Tyr210Asp
Trp112Pro  +  Val115Pro  +  Pro214Glu
Val115Glu  +  Gly139Asn  +  Tyr210Gln
Gly 69Ser  +  Thr 73Ser  +  Tyr210Pro
Ala114Ser  +  Ser207Asp  +  Thr224Pro
Thr 73Pro  +  Asn166Glu  +  Thr212Gly
Gly 72Gln  +  Gly110Asp  +  Leu221His
Tyr210Cys  +  Pro214Asn  +  Tyr218Asp
Gly 69Asn  +  Gly136Pro  +  Asn166Ser
Thr 73Gly  +  Gly108Ser  +  Ser220Glu
Thr 73Gly  +  Pro205Ser  +  Thr224Ser
Val103Met  +  Ala164Asp  +  Leu221Met
Thr111Asn  +  Thr167Glu  +  Phe193Met
Thr 73Asp  +  Tyr210Asn  +  Thr217Gln
Asn 66Glu  +  Thr 73Gly  +  Leu221Gln
Thr113Asp  +  Asn166Ser  +  Thr167Pro
Asn 66Gln  +  Tyr171Gly  +  Tyr210Pro
Asn 68Ser  +  Gly 69Pro  +  Asn170Gln
Asn 68Ser  +  Val115Glu  +  Tyr210Asn
Thr113Gln  +  Ser191Asp  +  Leu221His
Thr167Pro  +  Trp208Val  +  Tyr210Leu
Gly162Glu  +  Trp208Ala  +  Thr217Pro
Leu104Met  +  Ser207Asp  +  Thr212Gly
Thr137Gln  +  Asn163Asp  +  Pro205Gln
Val115Thr  +  Thr217Gln  +  Ser220Asp
Ser 70Asp  +  Val138Ser  +  Tyr210Val
Pro214Gly  +  Leu221Ile  +  Thr224Asp
Leu134Val  +  Gly165Gln  +  Trp208Thr
Ser109Asp  +  Ala114Asn  +  Tyr210Ser
Asn 66Ser  +  Val115Asn  +  Trp208Gln
Thr 73Pro  +  Phe193Glu  +  Gly206Gln
Leu134Ser  +  Leu221Pro  +  Ser222Asp
Val115Cys  +  Ala164Glu  +  Asn170Gln
Ala164Asn  +  Trp208Cys  +  Thr217Glu
Asn 66Glu  +  Ala168Pro  +  Thr215Pro
Gly135Ser  +  Ser207Asp  +  Thr217Gln
Asn140Gln  +  Thr167Asp  +  Trp208Met
Gly165Gln  +  Trp208Thr  +  Leu221Asn
Thr 73Pro  +  Asn106Glu  +  Leu221Cys
```

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```
Thr113Ser  +  Leu221Ile  +  Gly223Asp
Asn 66Asp  +  Gly136Gln  +  Gly162Gln
Ser191Glu  +  Thr217Asn  +  Thr224Pro
Leu104Gln  +  Gly162Glu  +  Tyr210Gln
Trp112Gln  +  Asn163Asp  +  Thr217Gly
Leu134Pro  +  Thr217Asn  +  Leu221Glu
Gly165Pro  +  Tyr210Met  +  Thr217Glu
Ala164Gly  +  Ser191Glu  +  Trp208Cys
Val115Cys  +  Gly139Asn  +  Pro205Asn
Gly135Pro  +  Trp208Ala  +  Tyr210Pro
Pro169Asn  +  Thr195Glu  +  Tyr210His
Ser107Asp  +  Trp208Met  +  Tyr210Pro
Thr 73Gly  +  Ala164Gly  +  Tyr210Glu
Leu104Val  +  Pro205Gln  +  Leu221Asp
Thr111Gln  +  Ser141Asp  +  Leu221Asn
Trp112Cys  +  Thr167Gln  +  Thr217Pro
Val138Thr  +  Asn170Glu  +  Leu221Pro
Ser 70Asp  +  Leu134Ser  +  Thr224Asn
Thr137Asn  +  Ser207Asp  +  Tyr218Thr
Gly135Glu  +  Ala164Asn  +  Ala203Ser
Leu134Gly  +  Trp208Glu  +  Thr212Ser
Asn163Asp  +  Trp208Asn  +  Tyr210Thr
Val115Thr  +  Ser192Glu  +  Thr217Ser
Gly 72Pro  +  Ser109Asp  +  Tyr210Gln
Ser109Glu  +  Asn163Gln  +  Phe193Pro
Gly136Pro  +  Pro169Glu  +  Leu221Ser
Gly110Asn  +  Ser220Glu  +  Leu221Met
Trp112Gly  +  Tyr210Asp  +  Thr217Pro
Trp208Ser  +  Tyr210Ser  +  Leu221Pro
Trp112Met  +  Ser192Glu  +  Leu221Ala
Thr137Gly  +  Thr167Ser  +  Thr217Glu
Ser109Asp  +  Gly162Pro  +  Tyr210Ala
Ala114Thr  +  Gly162Asn  +  Ser191Glu
Thr195Asn  +  Tyr218Pro  +  Leu221Glu
Thr 73Glu  +  Ala164Gln  +  Pro214Ser
Asn 68Glu  +  Trp208Tyr  +  Thr217Asn
Gly165Pro  +  Thr212Ser  +  Ser220Glu
Gly 67Asn  +  Gly139Gln  +  Ala168Asp
Ser141Glu  +  Pro214Gln  +  Leu221Gln
Gly206Ser  +  Trp208Tyr  +  Thr215Gln
Leu134Pro  +  Gly162Asn  +  Phe193Ile
Trp112Ala  +  Thr217Asn  +  Ser220Asp
Thr111Gly  +  Gly139Glu  +  Thr212Asn
Ser107Glu  +  Tyr213Ala  +  Thr217Pro
Gly165Ser  +  Thr217Glu  +  Gly223Pro
Tyr210His  +  Thr217Asp  +  Leu221Ala
Trp208Cys  +  Thr217Asp  +  Tyr218Asn
Gly 69Gln  +  Val103Ala  +  Ser220Glu
Asn106Ser  +  Tyr210Gly  +  Gly223Ser
Trp208Cys  +  Tyr210Asn  +  Thr217Asp
Asn 66Gln  +  Gly139Gln  +  Thr217Gly
Thr 73Glu  +  Leu104Ile  +  Tyr210Ile
Thr113Ser  +  Pro169Asn  +  Thr217Ser
```

```
Tyr210Gly + Pro214Gly + Leu221Glu
Leu134Ile + Ala219Glu + Ser220Asp
Asn140Asp + Ser141Glu + Tyr218Ser
Tyr213Val + Ser220Glu + Leu221Glu
Tyr210Val + Ser220Glu + Leu221Asp
Ala164His + Ser220Glu + Leu221Glu
Tyr171His + Ser220Asp + Leu221Glu
Tyr210Ile + Ser220Glu + Leu221Asp
Trp112Leu + Ser220Asp + Leu221Glu
Gly136Pro + Ser191Asp + Ser192Glu
Ala168Ser + Thr217Asp + Tyr218Glu
Asn166Ser + Thr217Glu + Tyr218Asp
Gly162Asp + Asn163Glu + Leu221Met
Trp208Ser + Tyr210Glu + Ser220Asp
Ala114Thr + Gly165Asp + Phe193Glu
Ser207Glu + Thr217Asn + Ser222Glu
Asn140Ser + Trp208Glu + Ser220Asp
Trp208Asp + Leu221Asn + Ser222Glu
Gly139Gln + Trp208Glu + Leu221Asp
Asn 68Glu + Asp105Glu + Asn106Asp
Thr137Gln + Gly139Asp + Tyr171Glu
Trp208Asp + Tyr210Glu + Leu221Ala
Gly 69Pro + Asn166Glu + Ala168Glu
Tyr171Cys + Tyr210Glu + Leu221Asp
Thr137Glu + Pro169Asp + Asn170Ser
Gly162Glu + Tyr171Asp + Thr217Ser
Leu134Ile + Ser191Glu + Gly223Glu
Val115Pro + Ser207Glu + Ser220Asp
Gly139Pro + Ser207Asp + Ser220Glu
Ser 70Glu + Val103Pro + Asn106Asp
Gly 69Asp + Thr 73Asp + Tyr218Ile
Asn 66Asp + Trp208Gly + Pro214Glu
Asn 66Asp + Ser 70Glu + Gly136Ser
Asn163Asp + Ser191Asp + Ser207Glu
Asn 66Ser + Gly 69Asp + Ser109Glu
Gly 67Glu + Trp208Ile + Thr215Asp
Ser 70Glu + Tyr210Gln + Thr217Glu
Ser 70Glu + Gly135Pro + Thr217Glu
Val138Glu + Ser141Asp + Tyr210Gln
Val138Glu + Ser141Glu + Pro214Asn
Ser107Asp + Gly110Asp + Ala114Glu
Ser109Glu + Ala114Glu + Gly136Ser
Gly110Glu + Gly136Glu + Tyr210Gln
Gly135Ser + Tyr210Asp + Thr217Glu
Ser 70Glu + Pro169Asn + Leu221Asp
Val115Gly + Gly162Asp + Thr167Asp
Gly110Pro + Thr217Asp + Ser220Asp
Ala164His + Thr217Asp + Ser220Asp
Tyr210Cys + Thr217Asp + Ser220Asp
Val103Ser + Thr217Asp + Ser220Asp
Thr217Glu + Tyr218Val + Ser220Glu
Gly 69Ser + Thr217Glu + Ser220Glu
Tyr210Val + Thr217Asp + Ser220Glu
```

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```
Gly 67Glu  +  Ser107Glu  +  Thr111Asn
Asn166Asp  +  Ser191Asp  +  Leu221Val
Leu134Asp  +  Phe193Glu  +  Gly223Asp
Asn 66Asp  +  Ser216Asp  +  Thr217Glu
Val103Glu  +  Asn106Glu  +  Thr113Glu
Ser 70Asp  +  Gly139Ser  +  Ser220Asp
Leu104Asp  +  Asp105Glu  +  Ser191Glu
Ala114Glu  +  Ser216Asp  +  Thr217Glu
Thr195Glu  +  Ser216Asp  +  Thr217Glu
Val115Glu  +  Ser220Asp  +  Leu221Glu
Ser109Asp  +  Ser220Asp  +  Leu221Glu
Thr215Glu  +  Ser216Asp  +  Ser220Asp
Ser192Asp  +  Phe193Glu  +  Thr217Asp
Gly165Asp  +  Tyr210Glu  +  Ser220Asp
Ser 70Glu  +  Asp105Glu  +  Tyr218Asp
Gly 69Glu  +  Ala168Glu  +  Thr195Glu
```

TABLE 34

Multi-loop Quadruple Mutation Variants

```
Asn 66Gln  +  Leu104Gly  +  Tyr210Pro  +  Thr217Glu
Gly 67Ser  +  Gly 69Ser  +  Val103Gln  +  Gly223Asp
Trp112Cys  +  Ala164Glu  +  Trp208Gln  +  Thr217Gly
Leu134Ile  +  Trp208Gln  +  Ala219Gly  +  Ser220Glu
Leu104Ala  +  Ser109Glu  +  Thr111Gln  +  Val115Ala
Gly108Glu  +  Ala164Gly  +  Gly165Gln  +  Thr167Pro
Gly 69Glu  +  Asn106Gln  +  Trp112Cys  +  Thr113Pro
Thr 73Asp  +  Leu134Ser  +  Thr137Asn  +  Thr217Pro
Gly 67Ser  +  Gly136Pro  +  Thr195Ser  +  Leu221Ala
Thr 73Gly  +  Leu104Gly  +  Trp208Pro  +  Thr215Gly
Gly110Glu  +  Gly136Pro  +  Phe193Met  +  Thr217Pro
Ala164Gln  +  Tyr171Ile  +  Phe193Ile  +  Thr224Gly
Gly136Pro  +  Ala164Thr  +  Ser207Asp  +  Trp208Leu
Gly108Gln  +  Trp112Pro  +  Trp208Thr  +  Tyr210Glu
Thr111Asp  +  Leu134Val  +  Tyr210His  +  Thr217Pro
Gly136Gln  +  Trp208Phe  +  Ala219Asp  +  Leu221Met
Thr113Ser  +  Tyr171Leu  +  Trp208Val  +  Tyr210Thr
Gly136Ser  +  Ser141Glu  +  Trp208Thr  +  Tyr210Leu
Asn 66Ser  +  Thr 73Pro  +  Thr113Pro  +  Asn170Glu
Thr 73Pro  +  Gly139Ser  +  Tyr210Glu  +  Leu221Ala
Gly108Ser  +  Val138Ser  +  Trp208Leu  +  Thr217Ser
Asn 68Ser  +  Gly110Gln  +  Trp112Met  +  Ser192Asp
Thr 73Gln  +  Val103Ala  +  Gly162Ser  +  Ala164Glu
Thr137Asn  +  Asn140Asp  +  Phe193Thr  +  Trp208Ala
Leu134Ser  +  Asn163Gln  +  Ser220Glu  +  Gly223Asn
Asn140Glu  +  Tyr171Gln  +  Tyr210Val  +  Gly223Ser
Thr 73Pro  +  Leu134Val  +  Gly136Glu  +  Thr217Pro
Ser107Glu  +  Gly162Gln  +  Trp208Ser  +  Leu221Pro
Gly108Asn  +  Gly162Asn  +  Ser192Asp  +  Tyr210Cys
Val103Pro  +  Ser109Glu  +  Trp208Asn  +  Tyr210Pro
Thr 73Gln  +  Trp112Tyr  +  Leu134Cys  +  Asn170Ser
Gly 69Asn  +  Gly162Asn  +  Asn166Ser  +  Leu221Asn
```

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```
Gly 67Ser  +  Leu134Ser  +  Gly162Asn  +  Ala164Gln
Leu134Ile  +  Gly135Pro  +  Ala168Thr  +  Trp208Met
Trp112Ser  +  Ala168His  +  Thr217Ser  +  Leu221Ala
Gly110Ser  +  Thr111Gln  +  Thr215Ser  +  Ser222Asp
Thr111Pro  +  Thr137Asn  +  Pro169Gln  +  Ala219Glu
Thr 73Glu  +  Asn106Ser  +  Trp208His  +  Leu221Thr
Asn 66Asp  +  Gly 67Pro  +  Gly 69Ser  +  Thr224Ser
Leu104Cys  +  Val115His  +  Ser191Asp  +  Tyr218Val
Leu134Glu  +  Thr195Asn  +  Trp208Ser  +  Leu221Ser
Val103Ser  +  Gly139Asn  +  Thr217Asn  +  Leu221Cys
Asn106Gln  +  Ala164Pro  +  Asn166Asp  +  Phe193Pro
Asp105Glu  +  Gly135Pro  +  Val138Ser  +  Thr217Pro
Gly 67Asp  +  Thr113Asn  +  Ala114Asn  +  Tyr210Cys
Asn166Asp  +  Thr195Gly  +  Tyr210Gly  +  Thr224Gln
Trp112Pro  +  Asn166Ser  +  Phe193His  +  Tyr218Ile
Gly 69Pro  +  Leu104Glu  +  Val115Ser  +  Ala164Ser
Trp112Pro  +  Val115Glu  +  Gly139Asn  +  Tyr210Gln
Gly 69Ser  +  Thr 73Ser  +  Tyr210Pro  +  Thr224Pro
Gly 72Gln  +  Thr 73Pro  +  Gly110Asp  +  Leu221His
Thr111Asn  +  Gly136Pro  +  Tyr210Thr  +  Tyr218Asp
Gly108Asp  +  Gly136Asn  +  Asn166Ser  +  Leu221Thr
Val103Met  +  Pro205Ser  +  Leu221Met  +  Thr224Ser
Gly 69Asn  +  Thr 73Asp  +  Trp208Gln  +  Tyr210Leu
Thr 73Gly  +  Asn166Ser  +  Thr167Pro  +  Leu221Gln
Asn 66Gln  +  Thr113Asp  +  Tyr171Gly  +  Tyr210Pro
Gly162Glu  +  Thr167Pro  +  Trp208Val  +  Tyr210Leu
Ser207Asp  +  Trp208Ala  +  Thr212Gly  +  Thr217Pro
Leu104Met  +  Thr137Gln  +  Asn163Asp  +  Pro205Gln
Ser109Asp  +  Leu134Val  +  Gly165Gln  +  Trp208Thr
Ala114Asn  +  Val115Asn  +  Trp208Gln  +  Tyr210Ser
Asn 66Glu  +  Ala164Asn  +  Trp208Cys  +  Thr215Pro
Gly135Ser  +  Ala168Pro  +  Ser207Asp  +  Thr217Gln
Thr167Asp  +  Trp208Met  +  Thr217Gln  +  Leu221Asn
Thr 73Pro  +  Asn106Glu  +  Thr113Ser  +  Leu221Cys
Gly162Gln  +  Ser191Glu  +  Thr217Asn  +  Thr224Pro
Gly 69Gln  +  Leu104Gln  +  Gly162Glu  +  Tyr210Gln
Leu104Gln  +  Tyr171Pro  +  Thr217Gly  +  Leu221Glu
Gly110Glu  +  Phe193Asn  +  Trp208Cys  +  Gly223Pro
Ala164Gly  +  Ser191Glu  +  Pro205Asn  +  Trp208Cys
Gly135Pro  +  Ser207Glu  +  Trp208Ala  +  Tyr210Cys
Thr 73Glu  +  Leu104Val  +  Pro205Gln  +  Leu221Cys
Trp112Cys  +  Val138Thr  +  Thr167Gln  +  Thr217Pro
Ser109Asp  +  Ala164His  +  Gly223Pro  +  Thr224Asn
Asn 68Gln  +  Tyr210Pro  +  Thr212Ser  +  Ser220Asp
Leu134Gly  +  Trp208Glu  +  Tyr210Met  +  Thr212Ser
Asn163Asp  +  Asn170Gln  +  Trp208Asn  +  Tyr210Thr
Trp112Gly  +  Trp208Ser  +  Tyr210Asp  +  Thr217Pro
Trp112Met  +  Ser192Glu  +  Trp208Ser  +  Tyr210Val
Trp112Leu  +  Ser220Asp  +  Leu221Ala  +  Thr224Asn
Leu104Met  +  Thr111Asp  +  Ala164Gln  +  Pro214Ser
Gly139Gln  +  Gly165Pro  +  Thr212Ser  +  Ser220Glu
Thr113Asp  +  Leu134His  +  Trp208Ile  +  Leu221Ala
Asp105Glu  +  Pro214Gln  +  Thr215Gln  +  Leu221Gln
```

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```
Leu134Pro + Gly162Asn + Gly206Ser + Trp208Tyr
Trp112Ala + Phe193Ile + Thr217Asn + Ser220Asp
Ser107Glu + Thr212Asn + Tyr213Ala + Thr217Pro
Gly 67Gln + Gly 69Gln + Thr113Asn + Ser220Glu
Asn 66Gln + Trp208Cys + Tyr210Asn + Thr217Asp
Thr 73Glu + Leu104Ile + Gly139Gln + Thr217Gly
Thr113Ser + Pro169Asn + Tyr210Ile + Thr217Ser
Leu134Pro + Thr167Gln + Tyr210Met + Ser220Asp
Thr 73Glu + Asn166Gln + Tyr171His + Leu221Ala
Val138Met + Asn170Gln + Thr217Asn + Ala219His
Gly136Gln + Ala219Ser + Ser220Asp + Thr224Gln
Gly 69Asn + Asn166Gln + Tyr210Glu + Thr217Gly
Thr 73Ser + Gly165Gln + Thr212Pro + Ser222Glu
Thr137Pro + Asn140Gln + Tyr171Gly + Ser220Glu
Tyr210Pro + Tyr213Ile + Ser220Asp + Leu221Pro
Val103Asp + Asn106Ser + Phe193His + Thr217Gly
Asn 68Asp + Thr113Asn + Val138Ala + Trp208His
Asp105Glu + Leu134Met + Gly165Ser + Leu221Gln
Asn 68Gln + Trp208Thr + Thr217Ser + Ser220Asp
Tyr171Asn + Thr195Asn + Tyr210Asn + Tyr218Asn
Asn 66Ser + Pro169Ser + Tyr210Cys + Gly223Glu
Ala114Gln + Phe193Glu + Tyr210Gly + Thr224Pro
Asn 66Ser + Gly136Ser + Ala164Asp + Ala168Gln
Leu104Gly + Gly162Pro + Ser192Asp + Leu221Asn
Gly108Asp + Gly162Pro + Trp208Thr + Gly223Asn
Asn 66Glu + Thr113Gly + Ala164Pro + Gly223Pro
Gly110Pro + Trp112Ala + Ser216Glu + Leu221His
Val115Met + Pro169Ser + Trp208His + Tyr210Asp
Leu104Ser + Ser109Asp + Gly110Pro + Thr195Gly
Leu104Asn + Thr137Glu + Trp208Ser + Leu221Val
Asn106Gln + Ala114Asp + Tyr171Thr + Leu221Pro
Pro205Asn + Tyr210Asn + Thr217Gly + Tyr218Leu
Leu104Pro + Asn106Ser + Thr137Asp + Thr217Pro
Gly110Ser + Thr137Gln + Asn170Glu + Thr217Asn
Thr113Gly + Phe193Met + Trp208His + Tyr213Ile
Asn166Gln + Ser207Asp + Thr217Ser + Tyr218Met
Gly162Asp + Phe193Pro + Trp208Tyr + Thr217Gln
Gly 69Ser + Ser107Glu + Trp208Met + Tyr210Leu
Val103Asp + Thr167Pro + Ile209Val + Tyr210Gln
Thr111Asn + Asn170Ser + Trp208Tyr + Thr212Gly
Ser107Glu + Thr137Pro + Gly162Ser + Trp208Cys
Val103Thr + Ala164Thr + Ala168Glu + Thr217Ser
Gly139Pro + Tyr210Asn + Leu221Pro + Thr224Pro
Leu134Ser + Ser141Glu + Tyr210Ala + Thr217Gln
Val103Asn + Leu134Ala + Tyr218Met + Thr224Asn
Asn 66Asp + Gly135Gln + Asn163Ser + Leu221Val
Gly 69Glu + Val115Pro + Thr137Pro + Trp208Ile
Gly 69Pro + Gly165Pro + Tyr210Ser + Leu221Ile
Asn 66Gln + Gly110Glu + Trp112Gly + Trp208Pro
Trp112Ala + Ala114Pro + Gly135Ser + Asn163Asp
Gly 67Asn + Ser207Glu + Tyr210Thr + Thr217Pro
Asn166Asp + Thr167Pro + Ile209His + Tyr210Val
Thr 73Ser + Asn106Gln + Tyr171Glu + Thr217Asn
```

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```
Val115Glu + Tyr210Ser + Thr217Gln + Ala219Gly
Leu104Pro + Thr195Gln + Trp208Tyr + Ser222Asp
Ser141Asp + Ala204Gln + Trp208Val + Tyr210Leu
Gly 67Ser + Gly 69Glu + Val103Gly + Tyr210Gln
Tyr171Met + Phe193Leu + Thr215Asp + Thr217Gln
Asn 68Gln + Asp105Glu + Trp208Phe + Leu221Ala
Leu104Asn + Val115Gln + Leu134Gln + Pro214Glu
Gly165Ser + Ser191Asp + Pro214Gly + Leu221Thr
Gly 67Ser + Asn 68Gln + Tyr210Val + Ser220Asp
Ser109Asp + Val138Gly + Trp208Ser + Thr217Pro
Thr111Gly + Gly136Pro + Ser141Asp + Leu221Ile
Trp112Asn + Gly162Ser + Ile209Cys + Thr224Gln
Val115Ala + Pro169Glu + Tyr210Val + Leu221Cys
Gly135Ser + Tyr210Leu + Thr217Glu + Leu221Pro
Asn 66Gln + Ser109Asp + Gly110Gln + Tyr218Thr
Thr 73Gly + Trp208Ile + Thr217Glu + Leu221Asn
Leu134Ser + Trp208His + Tyr210Leu + Ser220Glu
Gly135Pro + Thr137Gln + Ala219Ser + Leu221Gln
Asn 68Gln + Asn106Glu + Pro169Gln + Thr215Gln
Val138Gln + Gly165Glu + Tyr218Leu + Leu221Cys
Phe193Gln + Ser207Asp + Trp208Cys + Thr217Gln
Gly 67Asn + Gly165Asn + Phe193Tyr + Pro205Ser
Asn 66Ser + Ala168Ser + Tyr210Cys + Thr217Asp
Pro214Gly + Ser216Asp + Thr217Gln + Leu221His
Asn 66Gln + Val115Gln + Asn166Asp + Tyr218Met
Thr111Glu + Gly165Ser + Ala219His + Leu221Thr
Ser141Asp + Asn163Ser + Phe193Tyr + Ala204Ser
Gly108Pro + Asn163Ser + Gly165Asp + Tyr213Ser
Trp208His + Tyr210Thr + Tyr218Gln + Ser220Asp
Gly 69Glu + Ala164Pro + Trp208Val + Thr224Ser
Ser109Asp + Ala203Pro + Trp208His + Thr217Ser
Thr 73Asp + Tyr171Cys + Tyr210Pro + Leu221Pro
Gly162Pro + Tyr218Val + Ala219Thr + Ser220Glu
Asn170Gln + Ser192Glu + Trp208Pro + Tyr210Gly
Thr113Ser + Thr137Gln + Phe193Ser + Pro205Ser
Trp112Pro + Thr137Gly + Ser220Asp + Leu221Asn
Gly162Glu + Ala164Ser + Ala168His + Pro214Asn
Ala114Pro + Leu134Glu + Asn140Ser + Phe193Ile
Ala164Gln + Ala168Glu + Trp208Asn + Tyr210Leu
Val103Pro + Gly139Pro + Asn163Asp + Gly165Gln
Gly162Ser + Trp208Glu + Tyr210Ser + Leu221Gln
Ser141Asp + Tyr171His + Trp208Ala + Leu221Met
Asn 66Ser + Gly139Gln + Asn163Gln + Tyr210Thr
Leu134Asp + Gly136Ser + Asn163Gln + Trp208Val
Ser191Asp + Phe193Leu + Pro214Gln + Thr217Ser
Gly 67Asn + Gly108Asp + Thr195Asn + Thr215Pro
Ala114Thr + Asn140Glu + Asn166Ser + Ala203Pro
Ser191Glu + Ala203Gly + Trp208Asn + Tyr210Ala
Asn140Asp + Ser141Glu + Ala164Gln + Tyr218Ser
Thr 73Ser + Tyr213Val + Ser220Glu + Leu221Glu
Phe193Met + Thr215Gln + Ser220Glu + Leu221Glu
Thr113Ser + Trp208Leu + Ser220Glu + Leu221Glu
Thr 73Pro + Val115Ala + Ser192Asp + Phe193Glu
```

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Ala114Asn + Trp208Pro + Tyr210Glu + Ser220Glu
Trp208Cys + Tyr210Glu + Ala219Asn + Ser220Asp
Ala114Pro + Gly139Pro + Tyr210Asp + Ser220Glu
Tyr210Glu + Thr217Gln + Tyr218Gln + Ser220Glu
Asn140Ser + Trp208Glu + Ser220Asp + Leu221Asp
Val103Pro + Trp208Glu + Tyr210Ser + Ser220Glu
Thr 73Gln + Ser107Asp + Ser109Glu + Asn163Gln
Gly 67Gln + Ser107Asp + Ser109Glu + Leu221Cys
Asp105Glu + Asn106Ser + Ser109Glu + Thr217Pro
Leu104Glu + Asp105Glu + Ser107Asp + Leu221Ser
Gly206Pro + Pro214Asp + Thr217Glu + Leu221Asn
Ser207Asp + Trp208Glu + Ser220Asp + Leu221Ser
Gly135Asp + Gly162Glu + Tyr171Asp + Thr217Ser
Gly 69Glu + Leu104Asp + Asp105Glu + Tyr210Ala
Ala168Asn + Ser220Asp + Leu221Pro + Ser222Asp
Phe193Gln + Ser207Glu + Thr217Ser + Leu221Glu
Gly110Gln + Gly165Gln + Ser222Asp + Thr224Asp
Asn106Glu + Ser109Asp + Phe193Ile + Trp208Leu
Gly110Asn + Thr137Glu + Pro169Asp + Asn170Ser
Gly136Glu + Gly162Glu + Trp208Ala + Thr217Asn
Leu134Ile + Ser191Glu + Leu221His + Gly223Glu
Gly139Pro + Tyr171Met + Ser207Asp + Ser220Glu
Val103Pro + Thr111Gly + Ser207Asp + Ser220Asp
Gly108Asn + Thr195Gly + Ser207Asp + Ser220Asp
Ser 70Glu + Val103Pro + Asn106Asp + Ala168Pro
Thr113Gly + Gly162Ser + Asn163Asp + Asn170Glu
Leu104Ile + Ser107Glu + Gly110Asp + Asn170Gln
Thr 73Gly + Gly135Asp + Pro169Asp + Trp208Thr
Tyr210Met + Thr215Asp + Tyr218Glu + Leu221Asn
Leu104Thr + Gly136Glu + Pro169Asp + Leu221Val
Asn 66Asp + Thr 73Glu + Tyr218Ile + Leu221Cys
Asp105Glu + Thr111Asp + Ala114Pro + Val138Met
Asn 68Glu + Thr 73Glu + Trp208Tyr + Thr217Asn
Ser207Glu + Tyr213Ile + Ala219Asp + Ser222Asp
Asn163Asp + Ala164Ser + Ser191Asp + Ser207Glu
Thr 73Glu + Ala168Ser + Tyr210Glu + Tyr218Glu
Gly 67Glu + Trp208Ile + Pro214Asn + Thr215Asp
Asp105Glu + Thr111Glu + Thr113Glu + Asn163Gln
Ser 70Glu + Tyr210His + Thr217Asp + Leu221Ala
Gly 67Glu + Gly108Asp + Thr137Gln + Thr167Pro
Ser107Asp + Gly110Asp + Ala114Glu + Tyr218Asn
Ser 70Asp + Tyr210Glu + Thr217Asn + Ser220Glu
Thr113Gly + Trp208Glu + Ser220Asp + Thr224Asp
Val138Met + Ala219Glu + Ser222Asp + Thr224Gln
Ser 70Glu + Trp112Tyr + Tyr218Cys + Leu221Glu
Gly 67Ser + Ser 70Glu + Pro169Asn + Leu221Asp
Val115Cys + Gly139Asn + Thr217Asp + Ser220Asp
Gly 69Ser + Val115Thr + Thr217Glu + Ser220Glu
Ala168His + Trp208Tyr + Thr217Asp + Ser220Glu
Thr111Gln + Gly135Pro + Thr217Asp + Ser220Glu
Ala164Thr + Ser191Glu + Trp208Glu + Thr215Asn
Asn 68Glu + Val103Glu + Ser109Glu + Asn166Gln
Ser 70Asp + Gly139Ser + Thr217Glu + Ser220Asp
```

```
Leu134Asp  +  Phe193Glu  +  Ala203Ser  +  Gly223Asp
Asn 66Asp  +  Asn 68Ser  +  Ser216Asp  +  Thr217Glu
Ala164Glu  +  Trp208Cys  +  Leu221Glu  +  Ser222Asp
Ser 70Glu  +  Ser207Glu  +  Ala219Ser  +  Ser220Glu
Gly 67Asp  +  Ser 70Asp  +  Thr 73Gly  +  Tyr210Glu
Trp208Ile  +  Thr217Glu  +  Tyr218Asp  +  Ser222Asp
Leu104Ile  +  Thr111Glu  +  Gly135Asp  +  Asn163Gln
Ala114Glu  +  Leu134Ile  +  Ala219Glu  +  Ser220Asp
Asn166Glu  +  Thr167Gly  +  Ser222Asp  +  Gly223Asp
Gly139Glu  +  Tyr210Ala  +  Ser222Asp  +  Gly223Glu
Val103Gly  +  Gly110Glu  +  Thr111Glu  +  Ser222Glu
Ala114Glu  +  Asn170Ser  +  Ser216Asp  +  Thr217Glu
Gly108Asp  +  Ser109Asp  +  Tyr210Gln  +  Thr217Glu
Asn 68Ser  +  Ser109Asp  +  Gly110Glu  +  Ser220Glu
Asn 68Glu  +  Asn163Ser  +  Ser207Asp  +  Trp208Glu
Ser207Asp  +  Trp208Glu  +  Thr217Glu  +  Leu221Cys
Gly 69Pro  +  Asp105Glu  +  Asn106Asp  +  Asn166Asp
Ser109Asp  +  Tyr210Gln  +  Ser220Glu  +  Leu221Asp
Tyr171Gly  +  Ser192Glu  +  Ser220Asp  +  Leu221Glu
Asn170Glu  +  Ser191Glu  +  Ser192Glu  +  Pro205Ser
Asn 66Asp  +  Tyr210Gln  +  Thr217Glu  +  Tyr218Glu
Asn106Asp  +  Ser107Glu  +  Thr137Asp  +  Tyr210Gln
Ser192Asp  +  Phe193Glu  +  Pro205Ser  +  Thr217Asp
Ser 70Asp  +  Gly162Asp  +  Asn163Glu  +  Leu221Ser
Gly 69Asn  +  Asn106Glu  +  Tyr210Asp  +  Ser220Glu
Val103Ser  +  Asn140Asp  +  Tyr210Glu  +  Ser220Asp
Gly 69Glu  +  Phe193His  +  Ser207Glu  +  Ser222Asp
Gly162Glu  +  Asn163Gln  +  Trp208Asp  +  Ser220Glu
Val115Cys  +  Asn170Asp  +  Trp208Asp  +  Ser220Asp
Gly110Glu  +  Trp208Asp  +  Leu221Asn  +  Ser222Glu
Pro169Asp  +  Tyr171Glu  +  Pro214Asn  +  Gly223Glu
Asn163Glu  +  Ser207Glu  +  Trp208Met  +  Tyr210Thr
Gly 67Glu  +  Ser 70Glu  +  Val115Cys  +  Gly162Glu
Gly162Glu  +  Ala164Glu  +  Ser220Glu  +  Leu221Gly
Asn 66Glu  +  Val103Asp  +  Gly108Asn  +  Leu221Met
Asp105Glu  +  Ser109Glu  +  Tyr218Asp  +  Leu221Ala
Gly 67Asp  +  Ala164Asp  +  Ala168Glu  +  Tyr213Asn
Gly 69Pro  +  Ser141Asp  +  Pro214Asp  +  Thr217Asp
Thr 73Glu  +  Trp112Ile  +  Trp208Gly  +  Ser220Asp
Gly 72Ser  +  Ser107Asp  +  Thr111Asp  +  Asn166Gln
Gly165Glu  +  Ser192Asp  +  Thr217Asp  +  Leu221Ile
Gly135Glu  +  Asn140Glu  +  Ala204His  +  Pro214Ser
Asp105Glu  +  Gly110Asp  +  Asn170Asp  +  Ala219Gly
Asn 66Asp  +  Asn 68Asp  +  Leu134Gly  +  Asn163Glu
Leu104Thr  +  Ala168Asp  +  Asn170Asp  +  Ser220Glu
Thr 73Asp  +  Ser109Glu  +  Pro214Glu  +  Thr217Asn
Thr 73Asp  +  Val115Cys  +  Pro214Asp  +  Thr224Glu
Ser109Asp  +  Thr113Asp  +  Leu134Asn  +  Phe193Pro
Gly 69Ser  +  Gly108Asp  +  Gly110Asp  +  Ser220Glu
Gly139Asp  +  Ser141Asp  +  Ala164Asp  +  Leu221Val
Asn 66Asp  +  Trp208Met  +  Ser220Asp  +  Ser222Glu
Trp112Asp  +  Tyr210Thr  +  Ser220Glu  +  Ser222Glu
Ser109Asp  +  Ser207Glu  +  Tyr210Gly  +  Leu221Glu
```

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```
Gly 69Asp  +  Asp105Glu  +  Thr137Asp  +  Trp208Asn
Ser109Asp  +  Thr111Glu  +  Ser191Glu  +  Tyr210Leu
Gly139Gln  +  Pro169Asp  +  Ser191Asp  +  Ser207Glu
Gly 67Ser  +  Asn140Asp  +  Ser222Asp  +  Thr224Glu
Val138Asp  +  Asn170Glu  +  Trp208His  +  Thr215Glu
Asn 68Asp  +  Thr111Gly  +  Val138Asp  +  Asn170Asp
Leu104Glu  +  Ser107Asp  +  Gly135Gln  +  Tyr171Asp
Leu104Pro  +  Trp112Asp  +  Asn140Glu  +  Ser222Glu
Gly139Glu  +  Asn170Asp  +  Gly206Pro  +  Ser220Glu
Ala164Asp  +  Asn170Asp  +  Ser216Asp  +  Thr217Pro
Thr113Glu  +  Ala114His  +  Ser207Glu  +  Ser220Glu
Gly110Glu  +  Val115Gln  +  Ser207Asp  +  Thr224Glu
Asn106Glu  +  Asn163Glu  +  Asn170Glu  +  Leu221His
Gly 69Asp  +  Thr 73Glu  +  Thr113Asn  +  Ser141Glu
Gly 69Asp  +  Thr 73Asp  +  Thr111Gly  +  Gly139Glu
Ser107Asp  +  Asn163Glu  +  Ser192Asp  +  Trp208Met
Asn 68Asp  +  Gly108Asp  +  Ser207Asp  +  Ile209Ala
Gly135Asp  +  Gly139Pro  +  Pro169Asp  +  Trp208Glu
Asn 66Glu  +  Thr167Gly  +  Trp208Glu  +  Pro214Asp
Asn 66Asp  +  Ser109Asp  +  Trp208Gly  +  Pro214Glu
Gly 69Glu  +  Asn140Ser  +  Ser192Glu  +  Pro214Asp
Leu104Gly  +  Gly139Glu  +  Ser191Asp  +  Thr224Asp
Asn106Gln  +  Thr113Asp  +  Asn163Asp  +  Asn166Glu
Leu134Glu  +  Ser207Asp  +  Trp208Pro  +  Tyr210Glu
Ser191Glu  +  Thr195Asn  +  Tyr218Pro  +  Leu221Glu
Asn170Asp  +  Phe193Ser  +  Ser220Glu  +  Thr224Asp
Asp105Glu  +  Ala114Glu  +  Ser192Glu  +  Thr224Asn
Gly 67Asp  +  Gly108Asp  +  Ser207Asp  +  Tyr210Cys
Asn 68Asp  +  Gly110Glu  +  Thr113Gly  +  Leu221Ala
Leu104Ile  +  Ala114Glu  +  Ser220Glu  +  Gly223Glu
Ser107Asp  +  Ser220Glu  +  Leu221Pro  +  Gly223Asp
Val103Asp  +  Val138Glu  +  Ser141Glu  +  Gly206Gln
Val138Glu  +  Ser141Asp  +  Tyr210Gln  +  Ser220Asp
Asn 68Asp  +  Val115Gly  +  Thr217Asp  +  Ser220Glu
Asn 66Asp  +  Leu104Gly  +  Ala168Thr  +  Ser216Glu
Val103Glu  +  Asn106Glu  +  Thr113Pro  +  Ser220Asp
Asp105Glu  +  Thr113Asp  +  Gly139Asn  +  Ser141Glu
Ser 70Glu  +  Asp105Glu  +  Gly139Asp  +  Tyr210Ala
Asn 66Glu  +  Tyr210Asp  +  Thr217Glu  +  Leu221Ala
Gly108Glu  +  Gly135Ser  +  Tyr210Asp  +  Thr217Glu
Gly 69Asp  +  Gly108Gln  +  Trp112Asp  +  Ser141Glu
Ser107Glu  +  Gly110Pro  +  Thr217Asp  +  Ser220Asp
Gly135Glu  +  Thr217Glu  +  Tyr218Val  +  Ser220Glu
Asn163Asp  +  Tyr171Thr  +  Thr217Asp  +  Ser220Asp
Ala114Thr  +  Thr137Glu  +  Thr217Asp  +  Ser220Asp
Asn166Glu  +  Thr212Pro  +  Thr217Asp  +  Ser220Asp
Gly 67Asp  +  Ser107Asp  +  Tyr210Asn  +  Ser222Glu
Asn140Ser  +  Trp208Gly  +  Thr217Glu  +  Leu221Asp
Asn166Gln  +  Thr215Pro  +  Thr217Glu  +  Leu221Asp
Val115Gln  +  Ala168Gln  +  Phe193Asp  +  Leu221Asp
Trp208Ile  +  Pro214Ser  +  Ser220Glu  +  Thr224Glu
Gly108Glu  +  Thr137Glu  +  Asn163Asp  +  Trp208Ser
Gly139Pro  +  Ala164Glu  +  Ser191Asp  +  Pro214Glu
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Thr 73Asp + Thr111Pro + Asn166Asp + Ser191Asp
Ser 70Glu + Ala168His + Thr195Glu + Ser216Asp
Ser109Glu + Thr137Asp + Gly139Ser + Ala164Gln
Asn170Gln + Tyr210Asp + Pro214Glu + Gly223Ser
Ser 70Glu + Gly139Asn + Ala164Glu + Ser220Asp
Asn 66Asp + Thr113Glu + Ser141Asp + Trp208Gly
Asn 66Asp + Gly108Glu + Thr215Gly + Leu221Ser
Asn 68Ser + Thr 73Glu + Asn166Glu + Leu221Asp
Thr 73Asp + Ser192Asp + Ser220Asp + Leu221Met
Ser107Asp + Thr111Asp + Ser192Glu + Thr217Ser

TABLE 35

Multi-loop Quintuple Mutation Variants

Asn 66Gln + Gly 67Ser + Leu104Gly + Tyr210Pro +
Thr217Glu

Gly 69Ser + Val103Gln + Trp112Cys + Thr217Gly +
Gly223Asp

Val115Pro + Leu134Ile + Ala164Glu + Phe193Asn +
Trp208Gln

Leu104Gly + Thr113Ser + Ala168Glu + Asn170Ser +
Ala219Gly

Gly 69Ser + Ser109Asp + Thr111Gln + Val115Ala +
Tyr210Pro

Val103Thr + Gly108Glu + Val115Pro + Tyr171Ser +
Trp208Asn

Leu134Ala + Val138Cys + Asn163Gln + Tyr171Ser +
Ser220Asp

Asn 66Ser + Val115Gly + Ser191Glu + Trp208Ile +
Thr217Ser

Gly 72Asn + Trp112Phe + Val115Ala + Asn166Gln +
Trp208Gly

Leu134His + Gly165Gln + Trp208Pro + Tyr210Val +
Thr217Glu

Leu104Pro + Tyr171Leu + Trp208Leu + Ala219Glu +
Leu221Ser

Asn166Glu + Thr167Pro + Tyr210Pro + Tyr213Ile +
Pro214Asn

Gly139Gln + Asn170Glu + Trp208Cys + Thr215Ser +
Leu221Asn

Ala114Glu + Gly135Ser + Ala168Thr + Ala203Thr +
Trp208Met

Ala164His + Ala168His + Phe193Ala + Thr217Ser +
Ser220Asp

Gly 67Pro + Gly 69Ser + Ser191Asp + Tyr218Val +
Thr224Ser

Gly139Asn + Ala164Pro + Phe193Pro + Thr217Asn +
Leu221Cys

Leu104Met + Ser207Asp + Trp208Ala + Thr212Gly +
Thr217Pro

Leu134Val + Trp208Thr + Pro214Gly + Leu221Ile +
Thr224Asp

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```
Ser109Asp + Ala114Asn + Val115Asn + Gly165Gln +
            Tyr210Ser
Leu104Ser + Gly110Pro + Gly136Glu + Ala168Thr +
            Ala219Gln
Gly110Pro + Leu134Pro + Asn166Asp + Asn170Ser +
            Trp208Asn
Thr113Gln + Pro169Gly + Asn170Gln + Tyr218Gln +
            Leu221Ser
Thr137Ser + Thr195Gln + Ser207Glu + Trp208Gln +
            Thr217Pro
Gly 69Gln + Val103Gly + Trp112Met + Trp208Asn +
            Pro214Gly
Gly136Pro + Gly162Ser + Trp208Ile + Tyr210Gly +
            Pro214Gln
Thr137Asp + Gly165Gln + Thr195Ser + Trp208Pro +
            Thr212Ser
Asn 66Ser + Pro169Ser + Tyr210Asn + Tyr218Asn +
            Gly223Glu
Ser 70Glu + Asn106Gln + Val138Ala + Ala168Gln +
            Leu221Val
Gly 69Ser + Thr113Asp + Ala114Gln + Trp208Thr +
            Ala219Ser
Thr113Gln + Ala168Asp + Pro169Ser + Asn170Ser +
            Ala219Thr
Gly139Ser + Asn170Gln + Tyr171Leu + Trp208Cys +
            Thr215Ser
Gly 67Ser + Ala114Asn + Gly139Ser + Tyr210Thr +
            Tyr218Ser
Trp112Glu + Ala164Pro + Tyr171Pro + Thr195Asn +
            Thr217Ser
Gly 67Glu + Val115Asn + Gly162Gln + Gly165Gln +
            Phe193Val
Gly135Ser + Tyr171Cys + Trp208His + Tyr210Met +
            Leu221Val
Gly 69Asn + Asn106Ser + Asn166Glu + Tyr210Cys +
            Leu221Gly
Gly135Ser + Tyr171Asn + Trp208His + Ser216Glu +
            Leu221Gly
Gly 69Asn + Ser109Asp + Gly165Asn + Phe193Asn +
            Trp208His
Pro169Ser + Ser207Glu + Pro214Asn + Thr215Asn +
            Thr217Gly
Ser 70Asp + Asn163Ser + Tyr210Val + Tyr213Asn +
            Leu221Ala
Gly108Gln + Gly110Ser + Gly135Gln + Thr137Asn +
            Trp208Glu
Asn 68Ser + Ala164Gln + Asn170Glu + Thr217Ser +
            Gly223Ser
Gly 69Ser + Trp112Cys + Ala164Gly + Leu221Ser +
            Gly223Glu
Gly 69Glu + Gly 72Gln + Thr137Pro + Trp208Ile +
            Tyr218Pro
Val103Gln + Val138His + Gly162Ser + Asn166Asp +
```

Trp208His

Val115Gln + Leu134Cys + Ala168Ser + Pro169Asp +
Tyr210Ser

Gly 67Gln + Trp112Leu + Phe193His + Thr195Glu +
Thr217Ser

Gly 67Gln + Leu04Asn + Ala164Glu + Ala204Ser +
Pro205Ser

Gly135Ser + Val138Ser + Asn140Glu + Tyr210Leu +
Thr217Gly

Gly 67Asn + Thr 73Ser + Tyr171Leu + Phe193Tyr +
Tyr210Pro

Gly135Pro + Trp208His + Tyr210Leu + Ala219Ser +
Leu221Gln

Asn 68Gln + Asn106Glu + Thr137Gln + Pro169Gln +
Thr215Gln

Thr137Asp + Val138Gln + Trp208Ile + Tyr218Leu +
Leu221Cys

Gly 67Asn + Gly165Asn + Phe193Tyr + Pro205Ser +
Trp208Cys

Asn 66Ser + Thr 73Ser + Ser107Asp + Ala168Ser +
Tyr210Cys

Thr137Gly + Tyr210Pro + Thr215Pro + Ser216Asp +
Thr217Ser

Thr 73Pro + Trp112Asp + Gly139Pro + Thr215Pro +
Leu221His

Leu04Cys + Thr111Pro + Trp208Pro + Leu221Cys +
Ser222Asp

Thr113Gln + Ala204His + Trp208Phe + Thr217Asp +
Leu221Cys

Thr111Gly + Gly139Gln + Tyr171Leu + Tyr210Met +
Thr217Glu

Gly110Asn + Val115Gln + Pro169Gln + Thr195Gly +
Ser220Asp

Tyr171Asp + Gly206Ser + Thr217Gln + Ala219Gly +
Leu221Thr

Ser109Glu + Thr111Ser + Val115Cys + Val138Cys +
Tyr171Ser

Leu04His + Asn163Asp + Trp208Thr + Tyr210Cys +
Thr217Asn

Gly136Asn + Gly139Pro + Ser141Glu + Pro169Gln +
Ala219His

Gly 69Ser + Gly136Ser + Thr137Asn + Thr195Pro +
Ala219Glu

Asn106Asp + Gly162Ser + Asn163Gln + Pro169Gly +
Leu221Pro

Ala168Gly + Phe193Met + Pro205Asn + Tyr210Asn +
Leu221Asn

Gly 69Pro + Gly139Asp + Ala164Gly + Tyr213Ser +
Thr217Ser

Gly 72Ser + Leu04Gln + Asn166Glu + Pro169Asn +
Thr215Gly

Leu134Cys + Trp208Asn + Tyr210Ala + Tyr218Gly +
Thr224Gly

Leu104Gln + Thr195Asn + Trp208Val + Tyr210Pro +
Leu221Cys

Gly 67Pro + Thr 73Asn + Trp208Pro + Ala219Asn +
Ser220Asp

Thr 73Gln + Gly108Pro + Gly139Pro + Ser192Glu +
Gly223Gln

Asn 68Glu + Thr 73Gln + Pro169Gln + Tyr210Pro +
Leu221Met

Leu104His + Thr113Gln + Asn140Gln + Thr167Asn +
Phe193Asp

Gly 67Pro + Asn 68Ser + Gly139Asp + Thr215Gly +
Thr217Pro

Gly136Ser + Val138Met + Thr167Gln + Tyr218Asn +
Ser220Asp

Asp105Glu + Gly110Ser + Trp112His + Tyr210Gln +
Ala219Thr

Asn 68Asp + Asn106Gln + Ala164Ser + Thr195Gln +
Trp208Cys

Leu104Val + Leu34Ala + Thr137Asn + Asn170Asp +
Leu221Ile

Gly 67Ser + Leu34Met + Trp208Ala + Tyr210Ser +
Thr217Pro

Ala114Gly + Gly135Asp + Gly139Ser + Asn170Ser +
Thr217Ser

Thr111Asn + Phe193Ser + Pro205Gln + Trp208Thr +
Leu221Glu

Ser109Asp + Val138Asn + Phe193Ala + Trp208Cys +
Thr212Gln

Ser 70Asp + Asn166Ser + Ile209Pro + Tyr210Met +
Leu221Ser

Asn106Ser + Leu34Cys + Gly136Ser + Ala168Thr +
Tyr210Glu

Gly 67Gln + Leu34Gln + Gly135Asp + Phe193His +
Thr224Gly

Asn 68Ser + Leu104Ala + Gly162Asp + Tyr171Met +
Thr217Gly

Asn163Glu + Thr212Gln + Thr215Asn + Thr217Asn +
Leu221Met

Gly 69Gln + Asn106Gln + Ser207Glu + Trp208Thr +
Thr217Gly

Gly139Glu + Ala168His + Thr195Gly + Leu221His +
Thr224Asn

Gly 72Ser + Ala114Asn + Leu34His + Pro169Glu +
Trp208Tyr

Val138Ser + Ala164Asn + Tyr210Cys + Tyr218Leu +
Ser220Asp

Leu104Gln + Tyr171Glu + Trp208Gly + Thr217Gln +
Ala219His

Asn106Ser + Tyr171Gly + Phe193Gln + Thr215Gln +
Ala219Gln

Gly 72Ser + Gly108Asn + Trp112Ile + Asn166Glu +
Leu221Cys

Asn 68Ser + Leu104Thr + Thr215Ser + Ala219Thr +

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Thr224Asp

Val138Ser + Asn163Asp + Phe193Cys + Pro205Asn +
Thr217Ser

Gly 67Ser + Tyr210Pro + Tyr218Thr + Ser220Asp +
Leu221Ser

Gly135Asn + Gly136Gln + Gly162Ser + Tyr210Asn +
Pro214Asn

Thr195Ser + Trp208Pro + Tyr210Gly + Ala219Ser +
Leu221Ile

Asn 68Glu + Thr111Pro + Asn163Ser + Phe193Tyr +
Leu221Val

Gly 67Ser + Thr 73Pro + Asn170Glu + Tyr210Leu +
Leu221Val

Gly135Asn + Gly139Ser + Phe193Asp + Thr217Gly +
Leu221Gly

Gly136Gln + Gly139Gln + Gly165Ser + Tyr210Asp +
Thr217Pro

Ser141Glu + Gly162Pro + Thr167Gly + Tyr210Ile +
Thr217Ser

Leu104Pro + Gly110Pro + Gly136Pro + Pro205Asn +
Thr217Glu

Val115Ala + Ala164Thr + Ser192Asp + Trp208Leu +
Tyr210Met

Thr 73Asn + Val103Asn + Thr215Asn + Thr217Gln +
Ser222Asp

Val103Pro + Trp208Tyr + Ile209Cys + Tyr210Thr +
Thr217Gln

Trp112Phe + Ala114Ser + Pro169Gln + Ser220Glu +
Leu221His

Gly108Asn + Leu134Met + Tyr210Ser + Tyr213Ile +
Pro214Glu

Ala168Pro + Trp208His + Tyr210Ala + Ser220Glu +
Leu221Gln

Gly139Pro + Asn140Glu + Ser141Asp + Asn166Ser +
Trp208Thr

Val103Asn + Thr111Gly + Tyr210Gln + Ser216Asp +
Thr217Glu

Val138His + Gly139Asn + Ser207Asp + Trp208Glu +
Leu221Gln

Ala168Asn + Pro169Gly + Thr217Pro + Ser220Asp +
Leu221Asp

Tyr210Leu + Ala219Gly + Ser220Asp + Leu221Asp +
Gly223Ser

Val103Ala + Pro169Asn + Thr217Gln + Ser220Asp +
Leu221Asp

Thr 73Asn + Asn170Asp + Tyr171Glu + Tyr210Ile +
Leu221Ser

Gly136Ser + Ser191Asp + Ser192Glu + Tyr218Cys +
Leu221Asn

Thr 73Pro + Val115Ala + Ser192Asp + Phe193Glu +
Trp208Gln

Thr137Gln + Gly162Asp + Asn163Asp + Ala203His +
Pro205Gln

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Asn163Gln + Tyr210Asp + Thr215Pro + Ser220Glu +
Gly223Pro

Asp105Glu + Asn106Glu + Ser107Asp + Trp208Phe +
Tyr210Ser

Trp112Gly + Phe193Gln + Ser207Asp + Trp208His +
Ser222Asp

Gly108Asn + Ser207Glu + Tyr213Thr + Thr215Pro +
Ser222Asp

Leu134Met + Ser207Asp + Trp208Val + Tyr210His +
Ser222Asp

Gly 69Glu + Asn106Asp + Ala114His + Ala168Ser +
Thr217Gln

Leu104Pro + Trp208Glu + Thr212Asn + Thr215Ser +
Ser222Asp

Thr111Pro + Thr137Asn + Pro169Gln + Tyr210Glu +
Ala219Glu

Thr 73Gly + Val115Gly + Trp208Glu + Tyr210Asp +
Ser220Glu

Gly135Gln + Gly136Glu + Asn170Ser + Tyr171Glu +
Trp208Gln

Thr 73Ser + Ser107Asp + Ser109Asp + Gly136Pro +
Tyr210Gly

Leu134Pro + Pro169Gln + Pro214Glu + Ser216Glu +
Thr217Gly

Asp105Glu + Ser109Glu + Pro169Gly + Tyr171Gln +
Thr212Ser

Gly135Pro + Val138Ala + Ser207Asp + Tyr210Thr +
Gly223Glu

Gly135Pro + Ser207Glu + Trp208Ala + Tyr210Pro +
Gly223Glu

Pro169Gln + Phe193Val + Tyr210Glu + Thr215Asn +
Leu221Asp

Ala114Gln + Gly136Ser + Ala219Asn + Ser220Asp +
Ser222Asp

Leu134Ser + Thr167Gly + Ser220Asp + Leu221Pro +
Ser222Asp

Gly108Gln + Trp112Leu + Tyr218Glu + Ser220Asp +
Leu221Glu

Gly108Pro + Ala114Asn + Ser207Asp + Tyr210Glu +
Ser220Glu

Thr137Glu + Gly162Glu + Pro169Asn + Asn170Ser +
Tyr171Asp

Asn 68Gln + Gly162Glu + Ala164Thr + Gly165Asp +
Tyr171Ala

Thr137Pro + Gly139Asp + Asn170Glu + Trp208Met +
Tyr210Thr

Asn 68Gln + Val138Ser + Gly162Pro + Ala164Glu +
Asn170Asp

Asn 68Gln + Ser207Glu + Trp208Thr + Thr217Ser +
Ser220Asp

Val138Ala + Phe193Thr + Ser207Asp + Tyr210Met +
Ser220Asp

Phe193Ala + Trp208Glu + Ile209Ser + Leu221Cys +

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                       Gly223Glu
   Thr111Gly + Phe193Gln + Ser207Asp + Ser220Asp +
                       Gly223Glu
   Ser 70Asp + Asp105Glu + Gly108Glu + Thr111Ser +
                       Tyr210Val
   Gly136Glu + Gly162Asp + Gly165Ser + Ala168Pro +
                       Thr195Glu
   Leu104Gly + Gly165Glu + Pro169Glu + Thr217Asn +
                       Leu221Ala
   Thr195Asn + Tyr210Asp + Thr217Glu + Ser220Glu +
                       Gly223Pro
   Trp208Phe + Tyr210Asp + Tyr213Cys + Thr217Glu +
                       Ser220Glu
   Ser 70Asp + Gly108Glu + Tyr171Gly + Thr217Gln +
                       Leu221Pro
   Thr195Gly + Ser207Glu + Tyr210Glu + Tyr213Cys +
                       Ala219Ser
   Asn 68Glu + Asp105Glu + Gly110Asp + Thr111Pro +
                       Asn140Ser
   Ser 70Asp + Val103Gln + Pro214Gln + Ser220Asp +
                       Leu221Glu
   Asn 66Asp + Thr 73Glu + Asn106Ser + Trp208His +
                       Leu221Thr
   Ala164Asp + Thr167Asp + Tyr171Pro + Ala219Gln +
                       Leu221Thr
   Ala114His + Ala219Gly + Ser220Glu + Ser222Glu +
                       Thr224Glu
   Asn106Asp + Ser109Asp + Thr111Asp + Thr137Ser +
                       Pro205Asn
   Leu104Cys + Asn163Glu + Ser192Asp + Ala219Pro +
                       Ser222Glu
   Gly162Asp + Ala164Gln + Ser207Asp + Trp208Tyr +
                       Gly223Asp
   Ser 70Asp + Ser107Glu + Ser109Glu + Val115Cys +
                       Gly135Ser
   Ala114Gly + Ala219Asp + Ser220Glu + Leu221Asn +
                       Gly223Glu
   Asn 66Asp + Asp105Glu + Val115Gln + Thr137Ser +
                       Trp208His
   Gly 69Glu + Thr 73Glu + Val103His + Ser107Asp +
                       Leu221Ala
   Gly108Pro + Trp112Gln + Gly162Asn + Ser220Glu +
                       Gly223Asp
   Asn 68Asp + Asn170Gln + Tyr171Pro + Thr215Asp +
                       Ser216Asp
   Ser 70Asp + Tyr171Ser + Thr195Gly + Tyr210Asp +
                       Ser220Glu
   Thr113Gly + Gly162Ser + Trp208Glu + Ser220Asp +
                       Thr224Asp
   Ser 70Glu + Asn140Ser + Ala203Gln + Ala219Glu +
                       Gly223Pro
   Trp112Pro + Asn170Ser + Ser216Asp + Thr217Asp +
                       Ser220Asp
```

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```
Thr 73Asn + Val138Asp + Pro169Glu + Asn170Ser +
              Thr217Gly
Ser 70Asp + Asp105Glu + Gly162Pro + Trp208Val +
              Thr217Ser
Gly 67Asn + Asn140Ser + Ala219Glu + Leu221Met +
              Ser222Glu
Gly 72Ser + Gly110Pro + Trp112Ala + Tyr210Asp +
              Thr217Glu
Gly108Pro + Trp112Leu + Leu134Cys + Gly135Asp +
              Ala164Asp
Asn 66Gln + Trp208Ala + Thr217Glu + Ser220Glu +
              Leu221Thr
Thr 73Gly + Leu134Ser + Thr217Glu + Ser220Glu +
              Leu221Asn
Gly 67Ser + Ala168Gly + Pro214Gln + Thr217Glu +
              Ser220Asp
Val115Cys + Gly139Asn + Tyr210Cys + Thr217Asp +
              Ser220Asp
Leu134Glu + Thr137Glu + Thr195Asn + Trp208Ser +
              Leu221Ser
Ala164Gln + Tyr210Val + Thr215Asp + Thr217Glu +
              Ser220Asp
Gly 67Asp + Ser 70Asp + Thr217Gly + Tyr218Asp +
              Leu221Ile
Ser 70Asp + Thr111Asn + Trp208Cys + Thr217Glu +
              Ser220Glu
Leu134Glu + Gly162Ser + Asn166Gln + Ser207Asp +
              Gly223Asp
Gly162Pro + Thr167Asn + Pro169Glu + Ser191Glu +
              Phe193Asp
Ser 70Glu + Trp112Tyr + Ser207Glu + Tyr218Cys +
              Leu221Glu
Asn 66Glu + Leu104Glu + Ser107Asp + Trp208Ser +
              Thr215Pro
Leu104Ala + Gly135Asp + Thr195Asp + Tyr213Ile +
              Thr217Gly
Thr167Gln + Ser192Glu + Ser207Glu + Trp208Cys +
              Ser220Asp
Gly165Pro + Ser191Asp + Trp208Gly + Ser220Asp +
              Leu221Glu
Gly135Glu + Asn163Asp + Tyr213Met + Leu221His +
              Ser222Asp
Thr111Asp + Gly136Glu + Gly162Glu + Ala164Asn +
              Pro214Ser
Thr113Gln + Tyr213Thr + Ser216Glu + Thr217Glu +
              Leu221Glu
Ser 70Glu + Gly108Glu + Ala164Gly + Gly165Gln +
              Leu221Asp
Ser 70Glu + Leu104Gln + Ser220Asp + Leu221His +
              Gly223Pro
Gly165Asp + Pro169Ser + Ser207Glu + Trp208Gly +
              Ser222Asp
Ala168Pro + Pro169Asn + Thr215Asp + Thr217Asp +
```

```
                              Leu221Glu
         Gly110Pro + Thr113Ser + Gly165Glu + Trp208Asp +
                              Ser222Asp
         Gly110Glu + Thr113Asp + Gly139Glu + Trp208Tyr +
                              Tyr210Ala
         Asn 68Glu + Asn106Glu + Tyr171His + Tyr210Gln +
                              Ser216Glu
         Ala164Glu + Ala168His + Ala219Glu + Ser220Asp +
                              Leu221Cys
         Asn 68Asp + Tyr210Gln + Ala219Glu + Ser220Glu +
                              Leu221Ser
         Thr 73Asp + Asn163Ser + Ala219His + Ser222Glu +
                              Gly223Glu
         Gly 67Asp + Asn 68Glu + Ala168Gln + Pro169Glu +
                              Phe193Asn
         Gly110Asp + Gly139Gln + Asn140Glu + Ser141Glu +
                              Tyr218Asn
         Ser109Glu + Ser216Glu + Thr217Asp + Tyr218Asn +
                              Leu221His
         Gly135Pro + Gly136Ser + Phe193Asp + Ser216Asp +
                              Thr217Glu
         Gly165Gln + Ser192Glu + Ala203Ser + Ser216Asp +
                              Thr217Asp
         Gly 67Gln + Leu134His + Gly136Asp + Ser207Asp +
                              Trp208Glu
         Val103Ser + Ser207Asp + Trp208Glu + Thr217Glu +
                              Leu221Cys
         Gly 69Pro + Asp105Glu + Asn106Asp + Asn166Asp +
                              Ile209Thr
         Trp112Tyr + Asn170Glu + Trp208Gln + Ser220Glu +
                              Leu221Glu
         Thr137Gly + Gly139Gln + Gly162Asp + Ser220Glu +
                              Leu221Asp
         Thr113Asp + Ala164Gly + Ser191Glu + Ser192Glu +
                              Thr195Gly
         Ser107Glu + Thr113Glu + Ala114Asp + Tyr210Pro +
                              Leu221Ala
         Asn 66Asp + Gly 67Asp + Leu134Pro + Tyr210Asn +
                              Ser222Asp
         Asn 68Gln + Leu104Ala + Pro169Asp + Asn170Glu +
                              Leu221Asp
         Asn106Glu + Ser107Asp + Gly139Asp + Ala203Asn +
                              Thr217Ser
         Thr 73Gly + Thr137Glu + Tyr210Asp + Thr217Gln +
                              Ser220Glu
         Trp112Ile + Gly136Glu + Phe193Thr + Tyr210Glu +
                              Ser220Glu
         Trp112Ala + Asn163Ser + Ser192Glu + Tyr210Glu +
                              Ser220Glu
         Ser 70Glu + Thr 73Ser + Val103Asp + Pro214Glu +
                              Tyr218Gly
         Asn106Glu + Ser109Glu + Thr195Gln + Trp208Pro +
                              Pro214Glu
```

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Asn 68Gln + Val103Asp + Thr137Gln + Ser207Asp +
Ser222Asp

Thr137Asp + Ser207Glu + Thr217Gly + Leu221Pro +
Ser222Asp

Gly 69Asp + Thr 73Glu + Thr111Pro + Gly139Asn +
Ser220Asp

Asn 66Glu + Ser 70Asp + Pro214Gly + Ser220Glu +
Leu221Ser

Ser 70Glu + Leu104Cys + Tyr210Ser + Thr215Glu +
Ser220Glu

Thr167Pro + Ser216Glu + Ala219Glu + Leu221Met +
Ser222Asp

Val138Asp + Asn166Glu + Thr167Asp + Ala168Thr +
Leu221Ala

Leu134Pro + Ser141Glu + Trp208Glu + Thr217Ser +
Ser220Glu

Gly135Glu + Tyr171Asn + Pro205Gln + Trp208Glu +
Ser220Asp

Trp112Asn + Thr167Glu + Ala168Gly + Trp208Asp +
Ser220Glu

Asn140Asp + Pro169Gln + Trp208Glu + Thr215Gln +
Ser220Glu

Asn106Gln + Gly136Asp + Trp208Glu + Thr217Ser +
Ser220Glu

Asn106Asp + Thr111Asn + Thr167Gly + Tyr210Asp +
Ala219Asp

Asn 66Gln + Pro169Asp + Tyr171Glu + Pro214Asn +
Gly223Glu

Trp112Gly + Asn163Asp + Thr167Glu + Ser191Glu +
Gly223Ser

Trp112Asp + Thr113Ser + Ala114Glu + Thr217Glu +
Gly223Gln

Asn 66Asp + Thr 73Gly + Val103Asp + Thr111Pro +
Thr195Asn

Asn 68Asp + Trp112Met + Trp208Asn + Pro214Asp +
Ser220Asp

Asp105Glu + Ser109Glu + Asn163Glu + Asn166Ser +
Tyr218Gly

Gly110Gln + Trp112Asp + Leu134Pro + Pro214Asp +
Thr217Asp

Gly 67Pro + Thr113Glu + Gly135Asp + Gly162Asp +
Thr215Gln

Ser 70Glu + Thr195Ser + Pro214Ser + Ser220Glu +
Gly223Glu

Gly110Pro + Val138Glu + Trp208Glu + Tyr210Glu +
Thr217Gly

Gly108Asn + Asn163Glu + Ala219Asp + Leu221Val +
Thr224Asp

Thr 73Asp + Gly108Asn + Ser109Glu + Pro214Glu +
Thr217Asn

Thr137Asp + Gly139Glu + Asn170Ser + Trp208Cys +
Tyr210Asp

Trp112Phe + Gly139Glu + Tyr213Thr + Ala219Glu +

Leu221Glu

Leu104Pro + Pro169Glu + Tyr210Glu + Leu221Asp +
Thr224Gly

Asp105Glu + Gly165Glu + Thr167Gln + Ala168Glu +
Tyr210Pro

Thr137Asp + Trp208Asn + Tyr218Ser + Ser220Asp +
Ser222Asp

Gly 69Glu + Thr113Gln + Thr217Ser + Ser220Glu +
Ser222Glu

Leu104His + Thr113Asp + Ser207Asp + Thr217Ser +
Leu221Glu

Ala114Thr + Gly135Asp + Ser207Asp + Thr217Ser +
Leu221Asp

Gly 69Ser + Leu104Asp + Asn106Glu + Ser191Glu +
Thr217Gly

Val103Ala + Tyr171Val + Ser207Asp + Ser216Asp +
Tyr218Asp

Gly 69Ser + Leu34Ile + Gly162Asp + Ser207Glu +
Ser220Glu

Gly 72Pro + Thr113Glu + Asn166Asp + Thr167Asp +
Thr217Gln

Asn166Glu + Thr167Asp + Ser207Glu + Thr217Gly +
Leu221Val

Thr111Asn + Trp112Glu + Val138Gly + Asn163Asp +
Tyr171Glu

Gly 67Pro + Asn106Glu + Leu134Pro + Thr137Asp +
Pro169Glu

Thr195Glu + Ser207Asp + Ser220Asp + Leu221Val +
Thr224Gly

Asn 68Asp + Gly 69Gln + Gly162Asp + Pro169Ser +
Gly223Asp

Thr 73Ser + Gly165Asn + Ser207Asp + Tyr210Asp +
Ser216Glu

Val103Glu + Ala164Asp + Asn170Glu + Trp208Ala +
Tyr210Ile

Ala114Thr + Ala164Asp + Asn170Glu + Tyr210Ala +
Thr215Asp

Asn 68Glu + Gly139Pro + Ala164Pro + Ser191Asp +
Pro214Glu

Asn 68Glu + Ala164Glu + Gly165Ser + Tyr210Gly +
Pro214Asp

Gly 67Ser + Thr167Gly + Ser207Glu + Ser216Asp +
Ser220Asp

Asp105Glu + Gly110Asn + Ser207Asp + Ala219Gly +
Ser220Glu

Val103Gly + Asn140Asp + Ser207Asp + Tyr213Asn +
Ser220Asp

Ser109Glu + Gly165Asn + Ser207Glu + Tyr210Cys +
Ser220Asp

Gly110Asn + Asn140Glu + Ser207Glu + Thr217Ser +
Ser220Glu

Gly110Asp + Gly136Pro + Gly206Gln + Ser207Glu +
Ser220Glu

Thr113Gln + Leu134Ile + Ser192Glu + Thr195Asp +
Ser220Asp
Gly136Glu + Trp208Glu + Thr217Gly + Leu221Asn +
Gly223Asp
Ser107Asp + Gly110Asp + Leu134Ser + Asn140Glu +
Tyr210Thr
Thr167Glu + Trp208Asp + Thr217Asn + Ala219Asp +
Gly223Asn
Gly 69Glu + Ala114Asp + Thr195Ser + Trp208Ser +
Pro214Glu
Val103Asp + Thr113Glu + Phe193Tyr + Trp208Asp +
Ala219Pro
Asn106Asp + Gly136Ser + Val138Met + Ser191Asp +
Ser222Asp
Gly135Asp + Gly139Glu + Tyr210Ala + Thr217Ser +
Ser222Asp
Thr113Asp + Asn140Ser + Ala203Gln + Ser207Glu +
Tyr210Glu
Gly108Glu + Thr113Gln + Ser207Glu + Tyr210Asp +
Pro214Asn
Gly135Glu + Asn163Asp + Tyr210Met + Tyr218Pro +
Ser220Asp
Asn163Asp + Ala164Thr + Ser191Glu + Thr217Asp +
Thr224Gln
Asn 68Glu + Leu104Asp + Val115Thr + Ala164Asp +
Pro214Gln
Thr 73Gly + Ser107Glu + Gly108Pro + Leu221Glu +
Thr224Asp
Gly 67Ser + Gly162Asp + Thr167Asp + Ser207Glu +
Thr217Ser
Gly 67Gln + Ser109Glu + Val138Ser + Pro214Glu +
Ala219Glu
Ser107Glu + Gly135Glu + Trp208His + Thr217Ser +
Thr224Glu
Asn 66Glu + Asp105Glu + Val138His + Ser192Glu +
Tyr210Ile
Asn 66Glu + Asp105Glu + Ala219Gln + Ser220Glu +
Leu221Asn
Gly 69Ser + Ser 70Glu + Gly110Glu + Thr217Asp +
Leu221Ala
Tyr171Glu + Ser192Asp + Ser207Glu + Trp208Pro +
Tyr210Met
Gly 67Gln + Asp105Glu + Ala164Asp + Pro169Asn +
Gly223Asp
Thr 73Asp + Ser141Asp + Trp208Leu + Ser216Glu +
Thr217Pro
Asn 66Asp + Trp208Gln + Ile209Thr + Tyr210Val +
Ser216Glu
Asn 66Ser + Val103Glu + Asn106Asp + Ser191Glu +
Tyr210Ala
Asn 68Gln + Leu134Glu + Ser192Glu + Leu221Gly +
Ser222Glu
Ser 70Asp + Asp105Glu + Gly108Ser + Gly139Glu +

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                              Thr217Gly
      Gly 67Ser + Asn106Asp + Gly165Gln + Ala219Asp +
                              Ser222Glu
      Asn106Gln + Leu34Met + Asn140Asp + Ala219Asp +
                              Ser222Asp
      Gly 69Glu + Val103Gln + Leu34Asp + Thr167Gln +
                              Trp208Gly
      Thr137Glu + Gly165Glu + Tyr210Ser + Thr217Ser +
                              Leu221Pro
      Ser107Asp + Gly162Asp + Thr167Asp + Tyr171Cys +
                              Trp208Ile
      Asn106Glu + Trp112Asp + Leu34Gly + Ser141Glu +
                              Phe193Ser
      Ala114Thr + Asn140Asp + Phe193Thr + Thr217Glu +
                              Ser220Glu
      Thr113Ser + Val115Asp + Leu34Val + Thr217Asp +
                              Ser220Glu
      Gly110Asp + Asn170Gln + Thr217Glu + Tyr218Ala +
                              Ser220Asp
      Thr167Asp + Phe193Thr + Thr217Asp + Ala219Gln +
                              Ser220Asp
      Gly108Glu + Pro214Asn + Thr217Glu + Ser220Asp +
                              Leu221Val
      Asn106Gln + Gly135Glu + Val138His + Thr217Glu +
                              Ser220Glu
      Gly110Glu + Tyr210Met + Thr217Glu + Ser220Asp +
                              Leu221His
      Gly 67Glu + Ser107Glu + Gly110Ser + Thr113Glu +
                              Thr215Ser
      Gly 67Glu + Ser107Glu + Thr111Pro + Ala168His +
                              Ser220Glu
      Thr111Asp + Gly136Asp + Trp208Glu + Tyr210Met +
                              Thr217Gln
      Asp105Glu + Gly162Ser + Asn166Asp + Ser191Asp +
                              Trp208Phe
      Asp105Glu + Asn166Glu + Ser191Glu + Trp208Ala +
                              Leu221His
      Gly 67Glu + Val103Glu + Ala164Asn + Ser207Asp +
                              Tyr218Ala
      Gly 72Asn + Leu04Asp + Asn106Gln + Thr113Asp +
                              Thr217Asp
      Ser 70Asp + Gly135Glu + Ala168Glu + Tyr210Ile +
                              Ala219Gly
      Asn106Gln + Ser109Glu + Thr113Asp + Thr137Glu +
                              Trp208Asn
      Ala114Ser + Val138Thr + Pro169Asn + Phe193Asp +
                              Trp208Glu
      Asn 68Asp + Val138Asp + Ala164Gly + Ala168Glu +
                              Thr217Gly
      Gly 69Asn + Val138Asp + Ala168Glu + Ser207Asp +
                              Thr217Pro
      Gly110Ser + Thr111Gln + Ala164Glu + Thr215Ser +
                              Ser222Asp
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Leu104Ser + Gly108Glu + Val115Glu + Val138His + Ser141Glu

Ser 70Glu + Ala114Ser + Thr137Ser + Ser207Asp + Ser216Asp

Gly 69Asp + Gly110Asp + Thr137Glu + Pro169Ser + Tyr171His

Ser 70Asp + Thr137Glu + Ala164His + Trp208Ile + Ser220Glu

Ser 70Glu + Thr137Glu + Thr217Asn + Ser220Glu + Gly223Asn

Gly110Gln + Trp112Cys + Val115Glu + Asn140Asp + Ser216Glu

Asn 68Asp + Ser109Glu + Asn166Gln + Trp208Pro + Leu221Asp

Thr113Pro + Thr167Glu + Tyr171Glu + Thr217Gln + Ser220Glu

Trp112Ile + Leu34His + Gly139Asp + Gly162Glu + Trp208Val

Gly110Asp + Gly162Gln + Trp208Ile + Tyr210Glu + Gly223Asp

Gly 67Asp + Thr111Asp + Trp208Val + Tyr210Ser + Ser216Glu

Gly 69Asn + Thr 73Glu + Asn106Asp + Ser141Glu + Gly162Asn

Gly 69Glu + Gly135Asn + Pro169Asn + Trp208Ala + Thr217Glu

Ala203Gly + Trp208Ala + Pro214Glu + Ala219Gln + Leu221Asp

Thr 73Gly + Gly110Glu + Pro214Asp + Ser220Glu + Leu221Asn

Val103Ala + Thr167Gly + Ser192Glu + Pro214Glu + Ser220Asp

Asp105Glu + Tyr171Gln + Pro214Asp + Thr217Ser + Ser220Asp

Tyr171Val + Ser191Asp + Trp208Pro + Pro214Glu + Ser220Glu

Gly 67Asp + Gly162Ser + Gly206Ser + Ser207Glu + Thr217Glu

Gly 67Glu + Trp112Phe + Ser207Asp + Thr217Asp + Ala219Gln

Asn 68Asp + Asn163Glu + Phe193Met + Gly206Ser + Leu221Glu

Asn 66Asp + Leu104Ala + Ser109Glu + Ala168Thr + Ser216Glu

Val138Met + Gly139Asp + Gly162Ser + Ala168Glu + Ser220Asp

TABLE 36

Multi-loop Sextuple Mutation Variants

Asn 66Gln + Gly 67Ser + Gly 69Ser + Leu104Gly + Tyr210Pro + Thr217Glu

Ser 70Asp + Gly110Pro + Gly136Gln + Phe193Ser +
    Trp208Cys + Leu221His
Thr113Pro + Gly139Asn + Ser141Glu + Tyr171Pro +
    Trp208Thr + Leu221Gly
Gly 69Gln + Leu104Thr + Ser107Asp + Leu134Thr +
    Thr215Ser + Leu221Gly
Leu104Met + Leu134Ala + Thr137Asp + Trp208Gly +
    Tyr210Val + Pro214Gly
Gly 69Ser + Thr 73Asp + Gly110Ser + Tyr210Leu +
    Thr215Gly + Thr217Ser
Asn 66Ser + Gly 72Gln + Thr 73Pro + Gly110Asp +
    Trp208Val + Leu221His
Thr111Asn + Ala164His + Thr167Glu + Phe193Met +
    Tyr210Asn + Thr217Gln
Asn 66Gln + Thr 73Gly + Thr113Asp + Asn166Ser +
    Thr167Pro + Tyr210Pro
Leu104Met + Pro205Gln + Ser207Asp + Trp208Ala +
    Thr212Gly + Thr217Pro
Leu34Val + Gly165Gln + Trp208Thr + Pro214Gly +
    Leu221Ile + Thr224Asp
Asn 66Ser + Ser109Asp + Ala114Asn + Val115Asn +
    Trp208Gln + Tyr210Ser
Asn106Gln + Ala164Pro + Gly165Ser + Tyr171Ala +
    Ser220Glu + Thr224Ser
Gly110Ser + Gly136Pro + Ile209His + Thr217Gln +
    Leu221Ile + Thr224Asp
Asn 66Gln + Val115Pro + Trp208Asp + Thr217Ser +
    Leu221Ile + Thr224Pro
Gly 67Glu + Thr 73Asn + Asn170Ser + Tyr210Ser +
    Thr212Gln + Gly223Pro
Val103Gly + Ala164Thr + Tyr210His + Ala219His +
    Ser220Glu + Leu221Gly
Thr 73Pro + Asn140Gln + Thr167Asp + Trp208Met +
    Thr217Gln + Leu221Asn
Ser109Asp + Ala164His + Tyr210Pro + Thr212Ser +
    Gly223Pro + Thr224Asn
Gly110Gln + Tyr210Pro + Tyr213Thr + Tyr218Met +
    Ser220Asp + Leu221His
Val138Pro + Ala168Gly + Pro169Gln + Tyr210Ser +
    Thr217Gln + Ser220Asp
Ala114Thr + Thr137Asn + Gly139Ser + Trp208Pro +
    Pro214Gln + Ser220Glu
Val103Met + Thr137Ser + Ala203Ser + Tyr210Thr +
    Leu221Met + Gly223Ser
Leu104Glu + Thr111Ser + Val115His + Gly165Asn +
    Tyr210Met + Ala219His
Gly110Pro + Leu34Pro + Asn166Asp + Asn170Ser +
    Trp208Asn + Thr217Asn
Thr167Glu + Pro169Gly + Asn170Gln + Trp208Gly +
    Thr215Ser + Leu221Ser
Thr111Ser + Thr137Ser + Thr195Gln + Ser207Glu +
    Tyr210Val + Thr217Pro
Asn106Asp + Gly136Pro + Gly162Ser + Trp208Ile +

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```
Tyr210Gly + Pro214Gln
Ser 70Asp + Gly165Gln + Phe193Asn + Thr195Ser +
    Trp208Pro + Thr212Ser
Gly 72Ser + Ala114Gln + Asn166Gln + Phe193Glu +
    Tyr210Gly + Thr224Pro
Asn 66Ser + Gly136Ser + Gly162Pro + Asn163Ser +
    Ala164Asp + Ala168Gln
Gly 67Pro + Asn106Gln + Ser191Asp + Trp208His +
    Tyr218His + Leu221Asn
Trp112Leu + Ala168Pro + Trp208His + Tyr210Ser +
    Thr217Gly + Ser220Asp
Asn 66Ser + Thr111Gly + Val115Gly + Ala168Ser +
    Asn170Gln + Pro214Gly
Gly110Ser + Trp112Val + Gly162Asn + Thr167Pro +
    Pro169Gln + Ser216Asp
Gly 69Asn + Asn106Ser + Gly139Glu + Asn170Ser +
    Tyr210Cys + Leu221Gly
Gly 69Asn + Ser141Asp + Phe193Asn + Tyr210Ile +
    Ala219Asn + Leu221Ser
Ser109Asp + Pro169Ser + Trp208His + Pro214Asn +
    Thr215Asn + Thr217Gly
Asn 68Asp + Gly162Gln + Ala164His + Trp208Pro +
    Tyr210Leu + Leu221Val
Val103Thr + Gly139Pro + Ala164Thr + Thr217Ser +
    Leu221Pro + Thr224Pro
Gly 69Glu + Gly 72Gln + Thr137Pro + Trp208Ile +
    Tyr218Pro + Gly223Gln
Gly 67Pro + Leu104Cys + Val138Met + Gly139Ser +
    Ala203Gln + Ala219Asn
Trp112Ser + Phe193Ala + Thr217Gly + Tyr218Glu +
    Leu221Met + Thr224Asn
Asn163Gln + Trp208Leu + Tyr210Ser + Thr217Glu +
    Ala219Gly + Gly223Pro
Val103His + Gly110Gln + Gly139Ser + Ser192Asp +
    Phe193Asn + Thr217Gln
Gly108Gln + Pro169Asn + Thr195Ser + Trp208Ala +
    Thr215Pro + Thr217Glu
Ser109Asp + Thr111Asn + Trp112Gln + Thr167Gln +
    Thr195Gly + Tyr210Asn
Gly 72Gln + Trp112Leu + Gly136Asp + Tyr171His +
    Tyr210Thr + Leu221Ser
Gly 69Glu + Ala164Pro + Trp208Val + Tyr210Thr +
    Tyr218Gln + Thr224Ser
Ala114Pro + Leu134Glu + Asn140Ser + Ala164Gln +
    Phe193Ile + Trp208Asn
Asn 66Ser + Val138Met + Gly139Asn + Asn163Gln +
    Gly165Gln + Tyr210Thr
Asn106Gln + Ser191Asp + Phe193Leu + Trp208Val +
    Pro214Gln + Thr217Ser
Ala114Thr + Asn166Ser + Tyr171Gly + Ser191Glu +
    Ala203Pro + Tyr210Ala
Asn 68Gln + Tyr171Asp + Phe193Tyr + Gly206Ser +
    Tyr210Val + Leu221Thr
```

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Leu104Ala + Ala114Ser + Asn163Asp + Thr195Gly +
Gly206Gln + Leu221Ala
Gly110Asn + Val115Asn + Trp208Cys + Tyr210His +
Ala219Thr + Gly223Asp
Asn 66Asp + Gly 69Asn + Val115Ala + Ala164Ser +
Trp208Gln + Thr217Gln
Val115Met + Thr137Ser + Gly162Asn + Asn166Glu +
Tyr171His + Pro214Ser
Asn106Gln + Gly110Gln + Trp208His + Thr217Pro +
Ala219Asp + Ser220Asp
Gly 67Asn + Gly 72Asn + Ala114Gly + Asn140Gln +
Ser216Asp + Thr217Asp
Gly 69Gln + Thr 73Ser + Pro169Gln + Trp208Ile +
Ser220Glu + Leu221Asp
Asn170Glu + Tyr171Asp + Phe193Ser + Trp208His +
Leu221Thr + Thr224Gly
Gly 67Ser + Gly 69Glu + Ser 70Glu + Val103Gly +
Tyr210Gln + Ala219Asn
Trp112Gly + Asn166Ser + Trp208Met + Tyr210Glu +
Ser220Glu + Thr224Asn
Leu34Pro + Thr195Gly + Trp208Glu + Tyr210Asp +
Thr217Ser + Ser220Glu
Leu104Gln + Trp112Gln + Tyr171Pro + Trp208Glu +
Thr217Gly + Leu221Glu
Asp105Glu + Gly108Asp + Gly136Gln + Thr167Gln +
Tyr171Gln + Leu221Val
Ser107Glu + Ser109Glu + Val138Met + Ala168His +
Pro169Gln + Phe193Ser
Gly 67Glu + Ser 70Glu + Trp112His + Tyr171Ile +
Trp208His + Tyr210Ala
Val103Pro + Trp208Glu + Tyr210Ser + Ala219Glu +
Ser220Glu + Ser222Asp
Ser191Asp + Ser207Glu + Tyr210Ala + Thr215Gly +
Thr217Gln + Gly223Ser
Asn106Glu + Ser109Asp + Ala168Thr + Tyr171Met +
Phe193Ile + Trp208Leu
Asn 68Ser + Thr137Gln + Ser191Asp + Ser207Asp +
Trp208Asp + Leu221Thr
Val103Gln + Val138His + Asn166Asp + Ser192Glu +
Trp208His + Leu221Gly
Val103Pro + Gly139Pro + Asn163Asp + Gly165Gln +
Ala168Glu + Tyr210Leu
Asn 68Ser + Thr113Gln + Leu34Ile + Ser191Glu +
Leu221His + Gly223Glu
Gly110Gln + Ser207Glu + Trp208Gln + Thr217Pro +
Tyr218Gly + Ser220Asp
Gly162Ser + Phe193Ala + Trp208Glu + Ile209Ser +
Leu221Cys + Gly223Glu
Ser 70Asp + Asp105Glu + Gly108Glu + Asn140Gln +
Trp208Leu + Thr217Gly
Gly110Asn + Thr137Glu + Pro169Asp + Asn170Ser +
Thr195Glu + Tyr210His
Asn 68Gln + Gly162Glu + Ala164Thr + Gly165Asp +

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Tyr171Ala + Ser192Asp
Val138Met + Gly139Asn + Asn170Gln + Tyr210Glu +
    Thr217Asp + Ser220Glu
Gly139Ser + Asn163Asp + Ala164Ser + Ser191Asp +
    Tyr210Gly + Leu221Ala
Ser 70Asp + Thr 73Asp + Thr167Ser + Trp208Ser +
    Thr217Asp + Ala219Asp
Asn 66Glu + Leu104Glu + Asp105Glu + Ser107Asp +
    Leu221Ser + Gly223Pro
Asn 66Glu + Thr 73Asp + Gly110Pro + Ala168Gly +
    Tyr218Val + Leu221Ile
Gly 67Asn + Asp105Glu + Asn106Glu + Thr111Glu +
    Gly165Asn + Thr217Gly
Asn 66Asp + Tyr210Ser + Pro214Glu + Thr215Asp +
    Thr217Gln + Tyr218Asp
Thr 73Asn + Ser192Glu + Ala203Thr + Ser207Asp +
    Trp208Glu + Leu221Glu
Val138Gly + Pro169Glu + Ser192Glu + Thr195Glu +
    Trp208Pro + Thr212Gln
Thr 73Asp + Gly108Asn + Leu134Ser + Tyr210Ala +
    Thr217Gln + Tyr218Glu
Thr 73Asn + Ala114Thr + Val138Ser + Gly139Asp +
    Ser141Glu + Asn170Glu
Gly108Gln + Gly135Pro + Gly162Asn + Ser220Glu +
    Leu221Ala + Gly223Glu
Val103Gln + Trp112Cys + Ala164Glu + Trp208Gln +
    Thr217Gly + Gly223Asp
Thr111Gln + Leu134His + Ser191Asp + Phe193Asp +
    Thr195Gln + Trp208Asp
Val103Ser + Leu104Ile + Gly110Glu + Gly136Glu +
    Ala164Asn + Tyr210Gln
Leu134Ser + Thr167Gly + Ser191Glu + Ser220Asp +
    Leu221Pro + Ser222Asp
Gly 69Gln + Trp208Asp + Pro214Glu + Ser220Asp +
    Ser222Glu + Gly223Asn
Ser107Asp + Ser109Glu + Trp112Pro + Gly135Asp +
    Ala168Thr + Thr212Gly
Asn140Ser + Thr167Asp + Trp208Glu + Thr217Asn +
    Ser220Asp + Leu221Asp
Gly135Asp + Gly162Glu + Tyr171Asp + Tyr210Ala +
    Thr217Ser + Ser222Asp
Val115Asp + Tyr210Gln + Ser220Glu + Leu221Asp +
    Ser222Asp + Gly223Ser
Ala114His + Asn166Asp + Tyr171Cys + Tyr210Glu +
    Ser220Asp + Leu221Asp
Val115Cys + Gly139Asn + Ala164Gly + Pro205Asn +
    Thr217Asp + Ser220Asp
Thr167Gln + Tyr210Ile + Thr215Gly + Thr217Asp +
    Tyr218Asn + Ser220Asp
Thr137Ser + Trp208Leu + Tyr210Leu + Thr217Glu +
    Ser220Asp + Leu221Gly
Thr111Asn + Gly135Ser + Trp208Asp + Tyr210Glu +
    Thr217Glu + Leu221Ala
```

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Thr113Asn + Ala114Gly + Thr137Asp + Thr167Asp +
    Trp208Gln + Gly223Asn
Ser 70Asp + Thr 73Glu + Asn163Asp + Thr167Pro +
    Pro214Glu + Thr217Gly
Gly135Glu + Tyr171Asn + Pro205Gln + Ser207Glu +
    Trp208Glu + Ser220Asp
Gly 69Asp + Asp105Glu + Gly108Asp + Ser207Asp +
    Trp208Thr + Gly223Asn
Thr113Gly + Ala114Pro + Tyr210Asp + Pro214Asp +
    Thr217Gln + Ser220Asp
Asn 68Glu + Ser 70Asp + Asp105Glu + Ala114Thr +
    Gly135Ser + Ser220Asp
Thr111Ser + Thr137Glu + Ser207Glu + Trp208Thr +
    Ser220Glu + Leu221Asp
Gly 69Asp + Leu104Asp + Asp105Glu + Asn140Glu +
    Asn166Ser + Leu221His
Thr137Asp + Gly139Glu + Asn170Glu + Trp208Cys +
    Tyr210Asp + Leu221Thr
Gly 67Pro + Trp112Gln + Ser192Asp + Tyr210Ile +
    Ser220Asp + Ser222Asp
Val103Gln + Gly108Asp + Gly135Glu + Thr137Glu +
    Ala168Gln + Tyr210Asn
Val115Asp + Leu34Glu + Asn166Gln + Asn170Asp +
    Tyr210Val + Thr212Pro
Asn 66Asp + Asn 68Ser + Thr167Gln + Ile209Leu +
    Ser216Asp + Thr217Glu
Val103Glu + Gly108Asp + Gly110Glu + Gly165Pro +
    Thr195Glu + Thr217Gln
Asn 66Asp + Ser 70Glu + Thr 73Asp + Ser109Asp +
    Gly136Pro + Tyr210Ile
Gly 69Asp + Ser 70Glu + Asp105Glu + Asn163Glu +
    Tyr210Met + Thr217Gln
Asn 68Gln + Leu34Glu + Ser207Asp + Trp208Pro +
    Tyr210Asp + Leu221Asp
Asn 66Ser + Val115Gly + Gly162Asp + Ser191Glu +
    Trp208Ile + Thr217Ser
Ser 70Glu + Tyr213Thr + Thr217Asn + Tyr218Gly +
    Ser222Glu + Gly223Asp
Trp112Met + Gly165Asp + Pro169Ser + Ser207Glu +
    Trp208Gly + Ser222Asp
Gly 69Ser + Ser109Asp + Gly110Glu + Ala114Gly +
    Gly136Asp + Phe193Asp
Ala164Gln + Gly165Pro + Ser192Asp + Phe193Asp +
    Ser207Glu + Thr217Asp
Asn 68Glu + Thr 73Asn + Gly162Gln + Trp208Gln +
    Ala219Glu + Ser220Asp
Ser 70Glu + Leu134Ser + Gly165Asp + Asn166Asp +
    Ala168Gly + Ile209Gly
Leu104Asp + Asp105Glu + Trp208Gly + Tyr210Glu +
    Tyr218Ala + Gly223Pro
Asn 68Asp + Gly 69Glu + Thr111Asn + Trp112His +
    Gly136Asn + Pro169Glu
Gly108Ser + Asn140Asp + Ser141Glu + Ala164Gln +

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Ser207Glu + Tyr218Ser
Leu104Cys + Gly162Pro + Ser207Glu + Ser216Glu +
    Thr217Asp + Ala219Thr
Ala164Asn + Asn166Asp + Gly206Gln + Tyr210Thr +
    Ser216Asp + Thr217Asp
Gly108Asp + Ser109Asp + Asn166Ser + Thr217Gln +
    Ser220Asp + Leu221Thr
Ser109Asp + Gly110Asp + Val138Ala + Tyr171His +
    Ala204His + Ser222Glu
Asn 68Gln + Thr137Gly + Pro169Asp + Ser207Glu +
    Trp208Glu + Tyr218Gln
Trp112Asp + Thr113Glu + Val138Gly + Ser191Glu +
    Trp208Pro + Leu221Ser
Leu104Gly + Asp105Glu + Asn106Glu + Gly206Ser +
    Trp208His + Tyr218Glu
Gly108Asp + Leu134Asn + Gly136Gln + Trp208Phe +
    Ser220Asp + Leu221Asp
Gly108Gln + Trp112Tyr + Asn170Glu + Trp208Gln +
    Ser220Glu + Leu221Glu
Gly 72Asn + Asn166Asp + Phe193Ser + Trp208Tyr +
    Ser220Glu + Leu221Glu
Asn 66Glu + Gly 67Glu + Thr111Asn + Thr215Gln +
    Thr217Asn + Ser220Glu
Asn106Asp + Ser107Asp + Gly165Asp + Phe193Val +
    Trp208Val + Leu221Thr
Leu104Val + Gly165Gln + Ser192Asp + Phe193Glu +
    Tyr210Glu + Leu221Cys
Gly136Gln + Pro169Ser + Ser192Asp + Phe193Asp +
    Trp208Leu + Ser220Glu
Ser107Asp + Val115Thr + Thr137Gln + Gly162Asp +
    Asn163Asp + Ala203His
Val103Met + Ser107Glu + Trp208Val + Thr215Glu +
    Ser216Glu + Gly223Ser
Val103Ser + Asn140Asp + Ser141Glu + Tyr210Glu +
    Ser220Asp + Gly223Asn
Asn163Asp + Asn170Glu + Thr195Asn + Ser207Asp +
    Tyr210Met + Tyr218Pro
Asn 66Asp + Gly139Gln + Tyr210Glu + Thr217Ser +
    Ser220Asp + Thr224Asn
Gly 69Asp + Asn106Asp + Gly110Asp + Gly165Asn +
    Pro169Ser + Thr217Asp
Phe193Ile + Thr215Ser + Thr217Glu + Ser220Asp +
    Leu221Pro + Gly223Glu
Val103Gly + Asp105Glu + Thr195Asn + Ser207Asp +
    Ser222Asp + Gly223Asn
Gly 69Asp + Asn140Gln + Ala164His + Ala168Ser +
    Ser207Asp + Ser222Asp
Asn 68Asp + Thr111Asn + Ala114Gly + Gly135Gln +
    Ser207Glu + Ser222Glu
Asn106Asp + Gly139Asn + Gly162Glu + Pro169Ser +
    Ser191Asp + Thr224Asp
Thr113Asp + Ser192Asp + Ser207Glu + Tyr213Thr +
    Thr215Pro + Ser222Asp
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Gly 69Pro + Asn163Asp + Thr195Glu + Trp208Ser +
   Tyr210Glu + Leu221Cys
Gly 69Pro + Ser 70Asp + Gly110Ser + Gly135Glu +
   Asn170Glu + Leu221Ile
Gly 69Glu + Ala164His + Thr195Gln + Ser220Glu +
   Leu221Glu + Thr224Glu
Gly 69Gln + Gly136Asp + Val138Asp + Pro169Gly +
   Asn170Gln + Ser220Glu
Gly136Glu + Val138Asp + Thr195Asn + Ser220Glu +
   Leu221Gly + Gly223Pro
Asn 68Gln + Ser107Glu + Ser109Glu + Gly139Gln +
   Ala164Gln + Ser207Asp
Ser107Glu + Ser109Glu + Pro169Gln + Tyr210Met +
   Thr217Pro + Ser222Asp
Gly 67Asp + Ser 70Glu + Val103Ala + Thr111Glu +
   Tyr210Asn + Pro214Ser
Asn 66Glu + Gly 67Asp + Leu134His + Ser191Asp +
   Ser207Glu + Trp208Thr
Asn 66Glu + Val103Asp + Gly108Asn + Asn170Ser +
   Thr217Asn + Leu221Met
Thr 73Glu + Trp112Pro + Asn166Ser + Phe193His +
   Tyr218Ile + Ser220Glu
Val115His + Ser207Glu + Trp208Tyr + Tyr210Leu +
   Pro214Glu + Leu221Glu
Asn 66Gln + Ser107Glu + Ser109Glu + Ser207Glu +
   Gly223Asp + Thr224Gly
Asn 66Glu + Asn 68Asp + Thr 73Gln + Thr195Ser +
   Ser207Asp + Tyr218Pro
Ser 70Asp + Leu104Ile + Asn163Asp + Thr217Gln +
   Leu221Val + Thr224Glu
Gly 67Asp + Val103Glu + Asn106Asp + Ala168Asn +
   Thr195Glu + Trp208Leu
Thr137Gly + Gly162Glu + Phe193Asp + Trp208Asp +
   Thr217Asn + Tyr218Val
Gly135Pro + Gly139Glu + Ser207Glu + Trp208Ala +
   Tyr210Cys + Gly223Glu
Asn106Ser + Thr137Asp + Gly139Glu + Ser141Asp +
   Ala204Gln + Trp208Glu
Trp112Ala + Asn163Glu + Ser192Glu + Trp208Val +
   Tyr210Glu + Ser220Glu
Gly110Asp + Trp112Asp + Tyr210Thr + Ser220Glu +
   Leu221Met + Ser222Glu
Gly 67Asp + Gly 69Asp + Thr 73Pro + Ser109Asp +
   Trp208Gln + Ser222Asp
Asn 66Ser + Val103Glu + Asp105Glu + Trp112His +
   Ser191Glu + Tyr210Ala
Gly 67Glu + Gly 69Asn + Gly162Glu + Ser207Glu +
   Thr217Asn + Gly223Glu
Ser 70Asp + Gly165Asp + Ala168Asp + Trp208Pro +
   Thr217Gly + Leu221Ile
Gly 67Glu + Val103Asn + Gly136Gln + Trp208Pro +
   Ser216Glu + Leu221Met
Gly 67Asn + Gly139Glu + Ser141Glu + Tyr213Gly +

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Ser220Asp + Leu221Asn
Gly 67Glu + Leu104Ser + Tyr210Ser + Ser220Asp +
    Ser222Glu + Gly223Gln
Gly 67Asp + Asn 68Glu + Gly136Asp + Ala168Gln +
    Pro169Glu + Phe193Asn
Asn 66Glu + Gly 69Asp + Asn140Gln + Ser192Asp +
    Phe193Asp + Tyr210Leu
Leu104Asp + Asn106Glu + Asn170Glu + Tyr210Ile +
    Thr215Ser + Thr224Pro
Leu104Asp + Ser107Glu + Gly135Asp + Gly139Asn +
    Ala164Glu + Thr217Gly
Trp112Glu + Gly139Glu + Ala164Glu + Thr195Glu +
    Thr212Ser + Tyr213Ser
Asn 68Asp + Thr111Gly + Thr113Ser + Val138Asp +
    Asn170Asp + Phe193Ser
Asn163Gln + Ala168Asp + Asn170Asp + Ser207Glu +
    Thr217Gly + Ser220Asp
Gly 67Asp + Asn106Glu + Thr113Ser + Gly136Gln +
    Asn166Glu + Leu221Cys
Asn 66Gln + Gly110Asp + Ala114His + Gly136Glu +
    Thr137Asp + Ser222Glu
Gly 67Asp + Asn 68Ser + Gly 72Pro + Gly139Ser +
    Thr167Glu + Thr195Asp
Asn 66Asp + Asn 68Gln + Ser191Glu + Ser207Asp +
    Trp208Val + Ser220Asp
Ser109Glu + Ala114Glu + Leu34Ile + Gly136Ser +
    Ala219Glu + Ser220Asp
Val115Glu + Ala168His + Pro169Asn + Tyr210Asp +
    Ser216Glu + Ala219Asp
Asn 66Glu + Asn106Asp + Asn170Ser + Trp208Thr +
    Ser216Asp + Tyr218Gly
Gly162Asn + Asn170Asp + Phe193Glu + Tyr210Gln +
    Ser220Glu + Thr224Asp
Gly108Ser + Leu34Pro + Thr137Asp + Pro169Glu +
    Ser207Glu + Thr217Gly
Thr137Asp + Val138Glu + Asn163Glu + Ala164Asn +
    Ala168Ser + Thr217Glu
Gly162Glu + Trp208Glu + Tyr210Val + Thr217Glu +
    Ser220Glu + Gly223Pro
Gly135Asp + Val138Thr + Ser207Asp + Thr217Pro +
    Tyr218Ile + Ser220Glu
Leu104Gly + Ser192Asp + Thr195Asp + Thr217Pro +
    Ser220Glu + Leu221Asn
Asn 66Ser + Gly136Glu + Val138Glu + Trp208Leu +
    Thr217Asp + Ser220Glu
Gly 69Ser + Val103Asp + Ser109Asp + Ser191Asp +
    Gly206Gln + Tyr210Pro
Ala114Gly + Gly162Asp + Pro214Gly + Thr215Asp +
    Thr217Glu + Ser220Glu
Thr137Gln + Gly139Asp + Gly165Pro + Tyr171Glu +
    Thr217Glu + Ser220Glu
Gly 67Glu + Asp105Glu + Asn140Glu + Phe193Gly +
    Trp208Leu + Leu221Cys

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Leu104Glu + Trp112Leu + Phe193Leu + Ser207Asp +
    Trp208Ala + Thr224Asp
Ser 70Glu + Thr113Gly + Gly162Ser + Asn163Asp +
    Ala164Glu + Ser220Asp
Gly 69Glu + Ser 70Asp + Thr111Glu + Ser220Asp +
    Leu221Pro + Gly223Ser
Asn 66Gln + Leu104Pro + Gly136Glu + Asn163Asp +
    Tyr171Ile + Leu221Glu
Ser 70Asp + Leu104Glu + Ala168Asn + Pro169Glu +
    Thr195Asp + Trp208Phe
Ser 70Glu + Gly162Glu + Trp208Glu + Thr217Gly +
    Leu221Thr + Ser222Asp
Gly 69Asn + Thr137Glu + Gly139Pro + Asn166Asp +
    Pro169Asp + Trp208Asp
Gly 69Glu + Leu104Glu + Val115Ser + Asn140Ser +
    Ser192Glu + Pro214Asp
Leu104His + Asp105Glu + Val138His + Asn163Asp +
    Ser192Glu + Thr217Asn
Ala114Asn + Asn163Glu + Asn170Ser + Ser192Asp +
    Ala204Asn + Thr217Asp
Asn 68Gln + Thr 73Asp + Ser109Glu + Trp208Phe +
    Pro214Glu + Ser220Asp
Val115Cys + Leu34Asp + Gly135Pro + Ala164Thr +
    Asn170Asp + Trp208Asp
Asn 66Glu + Gly 69Glu + Trp112Gln + Gly135Asn +
    Gly139Asp + Asn170Glu
Thr 73Gly + Ser107Glu + Gly135Asp + Pro169Asp +
    Trp208Thr + Leu221Asn
Gly 67Ser + Ser 70Glu + Gly136Asp + Pro169Asn +
    Leu221Asp + Gly223Asp
Ser 70Asp + Gly110Glu + Val138Ser + Tyr210Val +
    Leu221Asp + Gly223Asp
Asn 66Asp + Leu104Glu + Ser109Glu + Tyr210Asn +
    Thr217Asn + Ser220Asp
Asn 66Glu + Gly 67Asp + Asn163Glu + Ser207Glu +
    Thr215Gly + Leu221Thr
Gly 69Glu + Tyr210Glu + Thr215Asp + Thr217Gln +
    Tyr218Gln + Ser220Glu
Ser 70Asp + Thr137Glu + Ala164His + Thr167Gly +
    Trp208Asp + Ser220Glu
Val138Met + Gly139Gln + Asn140Glu + Ala168Pro +
    Tyr171Glu + Thr215Glu
Val103Gly + Asn106Ser + Ser107Asp + Thr111Asp +
    Ser222Glu + Gly223Glu
Gly 72Gln + Asn106Glu + Gly110Asp + Thr113Glu +
    Gly135Asn + Ala219Glu
Ser109Glu + Thr113Asp + Trp208Ile + Ser220Glu +
    Leu221Asp + Thr224Ser
Gly 67Asn + Ser 70Asp + Gly108Asp + Asn170Glu +
    Thr215Pro + Leu221Thr
Gly 67Asp + Ser107Asp + Thr167Gly + Tyr210Asn +
    Ser220Asp + Ser222Glu
Gly 69Asp + Thr 73Glu + Asn106Gln + Thr111Asn +
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Val138Glu + Thr217Asp
Thr111Asn + Ser191Asp + Thr195Asp + Ala204Thr +
    Ala219Ser + Ser220Glu
Ser109Asp + Ser191Glu + Tyr210Val + Thr215Asn +
    Leu221Gly + Ser222Glu
Thr 73Glu + Leu104Asp + Asn106Gln + Thr195Ser +
    Ser207Glu + Ser220Asp
Asn 66Asp + Thr 73Asp + Leu104Glu + Gly136Gln +
    Gly162Asp + Leu221Gly
Gly 72Asn + Leu104Asp + Asn163Asp + Tyr171Ser +
    Ser191Glu + Thr217Asn
Val115Asp + Gly136Gln + Tyr210Glu + Tyr218Cys +
    Leu221Asn + Ser222Asp
Thr 73Asp + Asn140Ser + Asn163Glu + Thr195Asp +
    Ser220Asp + Leu221Pro
Asn 68Glu + Leu104Asp + Pro169Asn + Trp208Val +
    Thr217Asn + Gly223Glu
Gly110Glu + Thr167Gln + Ser192Glu + Ser207Glu +
    Trp208Cys + Ser220Asp
Val103Gly + Gly136Glu + Asn140Asp + Ser207Asp +
    Tyr213Asn + Ser220Asp
Thr113Asp + Gly136Asp + Thr137Gln + Asn163Glu +
    Asn166Glu + Trp208Phe
Asn 66Glu + Gly 69Glu + Ser109Asp + Ser192Asp +
    Phe193Val + Tyr218Ala
Ser 70Glu + Ser141Asp + Asn163Ser + Trp208Met +
    Tyr210Pro + Thr215Asp
Asp105Glu + Gly110Asn + Thr111Glu + Leu134Ala +
    Ser141Glu + Phe193Tyr
Asn 66Glu + Gly 69Ser + Gly108Asp + Thr111Asp +
    Thr195Gly + Tyr210Leu
Gly165Glu + Tyr171Asn + Ser207Glu + Thr215Glu +
    Thr217Gly + Thr224Glu
Asn 66Gln + Thr 73Ser + Gly139Asn + Pro214Asp +
    Ser220Asp + Leu221Ile
Asn166Glu + Ser192Asp + Trp208Thr + Tyr210Gly +
    Thr215Asp + Ser222Glu
Asn 66Asp + Ser191Asp + Thr215Asn + Ala219Asp +
    Ser222Asp + Gly223Gln
Asp105Glu + Gly108Glu + Thr195Asn + Ile209Leu +
    Pro214Asp + Ser220Asp
Thr111Glu + Val138Asp + Asn163Ser + Tyr218Met +
    Leu221Glu + Gly223Asp
Gly 67Glu + Val138Glu + Ser141Glu + Trp208Ile +
    Pro214Asn + Thr215Asp
Trp112Leu + Thr113Gln + Gly165Glu + Thr167Asp +
    Thr217Glu + Ser220Asp
Gly 67Gln + Asn 68Asp + Gly 69Asp + Phe193Ala +
    Ser216Glu + Ser222Glu
Ser 70Asp + Val115Glu + Gly162Ser + Ala168Thr +
    Tyr210Gly + Thr217Asp
Ser 70Asp + Gly136Asn + Gly165Glu + Tyr210Asn +
    Thr217Asp + Leu221His
```

Thr 73Ser + Val103Asn + Ser109Asp + Ser192Asp +
   Ser207Asp + Trp208Ile

Asn106Asp + Ser192Glu + Phe193Pro + Ser207Glu +
   Thr217Gln + Tyr218Ile

Ser141Asp + Thr167Asp + Ala168Asp + Ser191Asp +
   Ala219Asn + Leu221Thr

Asn 66Gln + Ser141Asp + Asn170Ser + Thr217Gln +
   Ser220Asp + Gly223Asp

Thr 73Gly + Asp105Glu + Ala114His + Ser220Glu +
   Leu221Asn + Gly223Glu

Asn106Asp + Asn140Gln + Trp208Ser + Tyr210Cys +
   Ser220Asp + Gly223Glu

Gly 67Gln + Asp105Glu + Ala164Asp + Pro169Asn +
   Tyr218Leu + Gly223Asp

Trp112Val + Asn166Asp + Phe193Gly + Pro214Glu +
   Ser220Asp + Ser222Glu

Gly108Asp + Thr111Asn + Ser192Glu + Thr217Asn +
   Ala219Glu + Ser220Asp

Val103Ser + Val115Asp + Gly139Glu + Asn166Ser +
   Ser207Glu + Ser220Glu

Leu104Asp + Asp105Glu + Asn140Glu + Thr167Asp +
   Pro214Gly + Ala219Gly

Gly 67Asp + Asn 68Asp + Thr111Asp + Ala164Gln +
   Phe193Ser + Ser207Asp

Ser109Glu + Trp208Asp + Ser216Glu + Thr217Asp +
   Tyr218Asn + Leu221His

Ser141Asp + Ala168Glu + Tyr210Met + Ser216Glu +
   Thr217Asp + Tyr218Ala

Ser109Asp + Gly110Asp + Asn166Asp + Asn170Ser +
   Thr215Ser + Leu221Asp

Gly110Glu + Thr167Glu + Ala168Asp + Trp208Met +
   Ser220Asp + Thr224Gln

Asp105Glu + Asn106Asp + Gly165Asp + Thr195Ser +
   Tyr218Ile + Ser220Asp

Asn106Glu + Ser141Asp + Tyr218Ser + Ala219Thr +
   Ser220Asp + Leu221Glu

Thr 73Gln + Thr111Glu + Asn166Asp + Trp208Ser +
   Ser220Asp + Leu221Asp

Ser107Glu + Ala168Asn + Pro169Asn + Ser191Asp +
   Ser192Asp + Thr217Asp

Ala114Glu + Asn166Ser + Thr212Pro + Thr217Glu +
   Tyr218Asp + Thr224Asp

Asn106Glu + Ser107Asp + Gly139Asp + Ala203Asn +
   Thr217Ser + Ser220Asp

Thr113Asn + Gly139Ser + Ser141Glu + Thr215Glu +
   Ser216Asp + Ser220Asp

Gly 69Gln + Asn140Glu + Gly162Glu + Asn163Asp +
   Tyr210Gln + Ser220Asp

Asn 68Asp + Ala114Pro + Gly135Glu + Trp208Asp +
   Leu221Ile + Gly223Asp

Asn 68Asp + Ala164Glu + Trp208Cys + Tyr210Glu +
   Ala219Thr + Ser220Asp

Thr111Asp + Asn166Asp + Asn170Gln + Tyr210Asp +

Thr212Ser + Ser220Glu
Ser141Glu + Gly165Asp + Trp208Ile + Tyr210Asp +
    Thr217Gln + Ser220Asp
Asn 66Gln + Val103Asp + Gly108Pro + Thr137Glu +
    Tyr210Glu + Ser220Glu
Gly108Glu + Thr137Gln + Asn163Glu + Tyr210Glu +
    Ser220Asp + Leu221Cys
Gly 69Gln + Ser 70Glu + Gly135Glu + Asn163Asp +
    Thr217Gly + Ser220Asp
Asn106Glu + Asn140Glu + Asn163Glu + Asn170Glu +
    Thr195Asn + Leu221His
Asn106Gln + Asn166Asp + Ala168Asn + Asn170Glu +
    Ser207Glu + Ser220Asp
Asn 66Asp + Ser 70Asp + Gly110Gln + Gly136Asp +
    Pro214Gln + Ser220Asp
Asn 68Ser + Pro169Asp + Ser192Glu + Ala219Asp +
    Gly223Ser + Thr224Asp
Thr 73Pro + Gly135Ser + Phe193Glu + Gly206Gln +
    Tyr210Asp + Thr217Glu
Trp112Glu + Val115Gln + Asn170Gln + Thr217Asp +
    Tyr218Met + Ser220Asp
Gly110Asn + Val115Glu + Thr137Gln + Ile209Gly +
    Thr217Asp + Ser220Glu
Gly108Glu + Thr113Gln + Pro214Asn + Thr217Glu +
    Tyr218Gln + Ser220Asp
Gly110Ser + Thr111Gly + Thr113Glu + Phe193Pro +
    Thr217Glu + Ser220Asp
Asn166Glu + Trp208Ile + Tyr210Met + Thr217Glu +
    Tyr218Val + Ser220Asp
Ser 70Glu + Val103Glu + Val115Gln + Pro169Ser +
    Tyr210Ala + Ser220Asp
Asn 66Asp + Ser107Asp + Trp208Met + Tyr210Pro +
    Ser220Asp + Ser222Glu
Gly110Ser + Thr111Asp + Gly135Gln + Gly136Asp +
    Thr137Asn + Trp208Glu
Thr167Asp + Ala168Ser + Pro169Glu + Trp208Thr +
    Thr215Asp + Leu221Asp
Val115Gln + Ala168Gln + Pro169Gln + Phe193Asp +
    Tyr210Ser + Leu221Asp
Asn 66Glu + Gly136Pro + Asn140Gln + Ser191Asp +
    Trp208Asp + Leu221His
Gly136Glu + Gly139Glu + Ser192Asp + Ala203Asn +
    Trp208Asp + Leu221His
Gly 67Asn + Thr113Gln + Thr137Glu + Thr167Asp +
    Ala219Gln + Leu221Glu
Gly 69Asn + Thr 73Asp + Thr111Pro + Asn166Asp +
    Ser191Asp + Leu221Val
Val138Pro + Asn166Glu + Ser191Asp + Tyr218Asn +
    Ser220Asp + Leu221Gln
Asp105Glu + Gly139Asn + Gly165Glu + Trp208Met +
    Tyr218Thr + Gly223Asp
Gly110Asn + Thr111Pro + Ala114Asp + Asn170Asp +
    Trp208Asp + Tyr210Asp

Gly 67Gln + Gly 69Glu + Ser107Asp + Thr167Glu +
    Ala168Asn + Ser207Asp
Gly 69Glu + Gly165Glu + Trp208Cys + Tyr210Cys +
    Pro214Asp + Ser220Asp
Ser 70Glu + Gly 72Gln + Val103Thr + Ser109Glu +
    Thr113Glu + Ser220Asp
Gly 69Pro + Val115Glu + Tyr210Asn + Ser216Asp +
    Ser220Glu + Ser222Glu
Gly136Glu + Trp208Ser + Ser216Asp + Ser220Asp +
    Leu221Asn + Ser222Asp
Leu104Glu + Val115Pro + Gly162Ser + Asn166Asp +
    Ser220Asp + Ser222Asp
Gly 67Glu + Val138Asp + Thr217Ser + Ser220Asp +
    Leu221His + Ser222Asp
Asn 68Asp + Asn170Glu + Gly206Asn + Ser207Asp +
    Trp208Ile + Leu221Asp
Asn106Gln + Ser109Glu + Thr113Asp + Leu134Gln +
    Thr137Glu + Trp208Asn
Asn106Glu + Gly139Gln + Thr167Glu + Pro169Gln +
    Ser191Asp + Ser207Glu
Ser107Glu + Val115Asp + Gly165Asn + Trp208Thr +
    Tyr218Asp + Ser220Glu
Gly 72Asn + Asn106Gln + Gly139Asp + Pro169Glu +
    Trp208Pro + Ser220Glu
Val138Asp + Ser141Glu + Pro169Ser + Ser207Glu +
    Ala219Glu + Leu221Thr
Asn106Gln + Ala114Asp + Gly135Asp + Thr195Asn +
    Ser207Glu + Ser220Asp
Ser 70Asp + Asn166Glu + Asn170Gln + Ser216Glu +
    Thr217Gln + Thr224Gly
Gly 69Asp + Asn140Ser + Gly162Pro + Trp208Gly +
    Thr217Glu + Leu221Asp
Asn 68Asp + Ser107Asp + Ala114Gln + Gly162Asp +
    Thr217Pro + Ser220Glu
Ser 70Asp + Thr111Asp + Ile209Met + Thr217Gly +
    Ser220Glu + Leu221Asn
Ser 70Asp + Leu104His + Thr111Gln + Tyr171Ser +
    Phe193Asp + Ser220Glu
Thr113Gly + Gly139Pro + Ser141Glu + Tyr171Glu +
    Ser192Asp + Ile209Ser
Trp112Ala + Thr113Glu + Val138Gln + Ser141Asp +
    Ser216Asp + Leu221Ser
Gly 69Asn + Asn106Asp + Trp112Gly + Gly162Asp +
    Phe193Glu + Ser220Glu
Asn 68Ser + Val103Glu + Asn106Asp + Ala168Pro +
    Pro169Asp + Pro214Glu
Leu104Glu + Ala114Ser + Asn140Ser + Gly165Asp +
    Ser207Asp + Ser220Glu
Asn 68Asp + Ser109Glu + Gly139Glu + Ala204Ser +
    Leu221His + Gly223Asn
Thr111Gly + Val138Pro + Gly139Asp + Ser207Glu +
    Ser216Glu + Ser220Asp
Asn106Asp + Asn170Ser + Ser192Glu + Thr195Asp +

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    Trp208His + Ser220Glu
Asn106Asp + Gly162Asp + Asn166Gln + Thr167Asp +
    Pro214Asp + Ala219Thr
Val115Glu + Gly136Glu + Ser207Glu + Tyr218Gly +
    Ala219Pro + Leu221Pro
Gly136Ser + Gly162Asp + Asn163Gln + Ala164His +
    Ser216Asp + Ser222Glu
Asn 68Ser + Thr 73Glu + Asn166Glu + Asn170Gln +
    Tyr171Gly + Leu221Asp
Thr113Glu + Val115Cys + Thr137Glu + Ser191Glu +
    Thr217Ser + Thr224Glu
Gly 67Asp + Ser107Asp + Gly139Asp + Gly165Pro +
    Thr167Ser + Thr217Glu
Thr 73Gly + Gly108Asp + Thr137Glu + Gly162Glu +
    Tyr210Gln + Ser220Asp
Leu104Asp + Gly139Pro + Tyr171Asp + Ser191Asp +
    Ser222Glu + Thr224Ser
Thr113Asp + Pro169Glu + Tyr210Gln + Pro214Ser +
    Ser220Glu + Gly223Asp
Gly108Asp + Gly136Glu + Ser141Asp + Thr195Asp +
    Thr215Ser + Thr217Gln
Ser107Asp + Asn163Glu + Pro169Gly + Ser207Asp +
    Trp208Asn + Gly223Asn
Gly110Asp + Asn163Asp + Ser191Asp + Thr195Gln +
    Tyr210Glu + Thr217Pro
Val103Glu + Gly108Asp + Ala203Gly + Trp208Met +
    Ala219Asp + Thr224Glu
Thr 73Gln + Thr111Asp + Leu134Asp + Gly136Ser +
    Asn163Gln + Ser207Asp
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II.   Cleaning Compositions

In another embodiment of the present invention, an effective amount of one or more of the enzyme variants are included in compositions useful for cleaning a variety of surfaces in need of proteinaceous stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); denture cleaning compositions, unlimited in form (e.g., liquid, tablet); and contact lens cleaning compositions, unlimited in form (e.g., liquid, tablet).

The cleaning compositions also comprise, in addition to the Thermitase variants described hereinbefore, one or more cleaning composition materials compatible with the protease enzyme. the term

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"cleaning composition material", as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, granule, bar, spray, stick, paste, gel), which materials are also compatible with the Thermitase variant used in the composition. the specific selection of cleaning composition materials are readily made by considering the surface material to be cleaned, the desired form of the composition for the cleaning condition during use (e.g., through the wash detergent use). The term "compatible", as used herein, means the cleaning composition materials do not reduce the proteolytic activity of the Thermitase variant to such an extent that the protease is not effective as desired during normal use situations. Specific cleaning composition materials are exemplified in detail hereinafter.

As used herein, "effective amount of enzyme variant" refers to the quantity of enzyme variant necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. Preferably the cleaning compositions comprise from about 0.0001% to about 10% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 1%, more preferably still from about 0.01% to about 0.1%. Several examples of various cleaning compositions wherein the enzyme variants may be employed are discussed in further detail below. All parts, percentages and ratios used herein are by weight unless otherwise specified.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing compositions, oral cleaning compositions, denture cleaning compositions and contact lens cleaning compositions.

A.   Cleaning Compositions for Hard Surfaces, Dishes and Fabrics

The enzyme variants of the present invention can be used in a variety of detergent compositions where high sudsing and good insoluble substrate removal are desired. Thus the enzyme variants can be used with various conventional ingredients to provide fully-formulated hard-surface cleaners, dishwashing compositions, fabric laundering compositions and the like. Such compositions can be in the form of liquids, granules, bars and

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the like. Such compositions can be formulated as modern "concentrated" detergents which contain as much as 30%-60% by weight of surfactants.

The cleaning compositions herein can optionally, and preferably, contain various anionic, nonionic, zwitterionic, etc., surfactants. Such surfactants are typically present at levels of from about 5% to about 35% of the compositions.

Nonlimiting examples of surfactants useful herein include the conventional C_{11}-C_{18} alkyl benzene sulfonates and primary and random alkyl sulfates, the C_{10}-C_{18} secondary (2,3) alkyl sulfates of the formulas $CH_3(CH_2)x(CHOSO_3)^-M^+)CH_3$ and $CH_3(CH_2)y(CHOSO_3^-M^+)$ CH_2CH_3 wherein x and (y+1) are integers of at least about 7, preferably at least about 9, and M is a water-solubilizing cation, especially sodium, the C_{10}-C_{18} alkyl alkoxy sulfates (especially EO 1-5 ethoxy sulfates), C_{10}-C_{18} alkyl alkoxy carboxylates (especially the EO 1-5 ethoxycarboxylates), the C_{10}-C_{18} alkyl polyglycosides, and their corresponding sulfated polyglycosides, C_{12}-C_{18} alpha-sulfonated fatty acid esters, C_{12}-C_{18} alkyl and alkyl phenol alkoxylates (especially ethoxylates and mixed ethoxy/propoxy), C_{12}-C_{18} betaines and sulfobetaines ("sultaines"), C_{10}-C_{18} amine oxides, and the like. The alkyl alkoxy sulfates (AES) and alkyl alkoxy carboxylates (AEC) are preferred herein. (Use of such surfactants in combination with the aforesaid amine oxide and/or betaine or sultaine surfactants is also preferred, depending on the desires of the formulator.) Other conventional useful surfactants are listed in standard texts. Particularly useful surfactants include the C_{10}-C_{18} N-methyl glucamides disclosed in US Patent 5, 194,639, Connor et al., issued March 16, 1993, incorporated herein by reference.

A wide variety of other ingredients useful in detergent cleaning compositions can be included in the compositions herein, including other active ingredients, carriers, hydrotropes, processing aids, dyes or pigments, solvents for liquid formulations, etc. If an additional increment of sudsing is desired, suds boosters such as the C_{10}-C_{16} alkolamides can be incorporated into the compositions, typically at about 1% to about 10% levels. The C_{10}-C_{14} monoethanol and diethanol amides illustrate a typical class of such suds boosters. Use of such suds boosters with high sudsing adjunct surfactants such as the amine oxides, betaines and sultaines noted above is also advantageous. If desired, soluble magnesium salts such as $MgCl_2$, $MgSO_4$, and the like, can be added at levels of, typically, from about

0.1% to about 2%, to provide additionally sudsing.

The liquid detergent compositions herein can contain water and other solvents as carriers. Low molecular weight primary or secondary alcohols exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing surfactants, but polyols such as those containing from about 2 to about 6 carbon atoms and from about 2 to about 6 hydroxy groups (e.g., 1,3-propanediol, ethylene glycol, glycerine, and 1,2-propanediol) can also be used. The compositions may contain from about 5% to about 90%, typically from about 10% to about 50% of such carriers.

The detergent compositions herein will preferably be formulated such that during use in aqueous cleaning operations, the wash water will have a pH between about 6.8 and about 11.0. Finished products thus are typically formulated at this range. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When formulating the hard surface cleaning compositions and fabric cleaning compositions of the present invention, the formulator may wish to employ various builders at levels from about 5% to about 50% by weight. Typical builders include the 1-10 micron zeolites, polycarboxylates such as citrate and oxydisuccinates, layered silicates, phosphates, and the like. Other conventional builders are listed in standard formularies.

Likewise, the formulator may wish to employ various additional enzymes, such as cellulases, lipases, amylases and proteases in such compositions, typically at levels of from about 0.001% to about 1% by weight. Various detersive and fabric care enzymes are well-known in the laundry detergent art.

Various bleaching compounds, such as the percarbonates, perborates and the like, can be used in such compositions, typically at levels from about 1% to about 15% by weight. If desired, such compositions can also contain bleach activators such as tetraacetyl ethylenediamine, nonanoyloxybenzene sulfonate, and the like, which are also known in the art. Usage levels typically range from about 1% to about 10% by weight.

Various soil release agents, especially of the anionic oligoester type, various chelating agents, especially the aminophosphonates and ethylenediaminedisuccinates, various clay soil removal agents, especially ethoxylated tetraethylene pentamine, various dispersing agents, especially

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polyacrylates and polyasparatates, various brighteners, especially anionic brighteners, various suds suppressors, especially silicones and secondary alcohols, various fabric softeners, especially smectite clays, and the like can all be used in such compositions at levels ranging from about 1% to about 35% by weight. Standard formularies and published patents contain multiple, detailed descriptions of such conventional materials.

Enzyme stabilizers may also be used in the cleaning compositions. Such enzyme stabilizers include propylene glycol (preferably from about 1% to about 10%), sodium formate (preferably from about 0.1% to about 1%) and calcium formate (preferably from about 0.1% to about 1%).

1.   Hard surface cleaning compositions

As used herein "hard surface cleaning composition" refers to liquid and granular detergent compositions for cleaning hard surfaces such as floors, walls, bathroom tile, and the like.   Hard surface cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about .01% to about 5%, more preferably still from about .05% to about 1% by weight of active enzyme of the composition. In addition to comprising one or more of the enzyme variants, such hard surface cleaning compositions typically comprise a surfactant and a water-soluble sequestering builder. In certain specialized products such as spray window cleaners, however, the surfactants are sometimes not used since they may produce a filmy/streaky residue on the glass surface.

The surfactant component, when present, may comprise as little as 0.1% of the compositions herein, but typically the compositions will contain from about 0.25% to about 10%, more preferably from about 1% to about 5% of surfactant.

Typically the compositions will contain from about 0.5% to about 50% of a detergency builder, preferably from about 1% to about 10%.

Preferably the pH should be in the range of about 8 to 12. Conventional pH adjustment agents such as sodium hydroxide, sodium carbonate or hydrochloric acid can be used if adjustment is necessary.

Solvents may be included in the compositions. Useful solvents include, but are not limited to, glycol ethers such as diethyleneglycol monohexyl ether, diethyleneglycol monobutyl ether, ethyleneglycol monobutyl ether, ethyleneglycol monohexyl ether, propyleneglycol

monobutyl ether, dipropyleneglycol monobutyl ether, and diols such as 2,2,4-trimethyl-1,3-pentanediol and 2-ethyl-1,3-hexanediol.   When used, such solvents are typically present at levels of from about 0.5% to about 15%, preferably from about 3% to about 11%.

Additionally, highly volatile solvents such as isopropanol or ethanol can be used in the present compositions to facilitate faster evaporation of the composition from surfaces when the surface is not rinsed after "full strength" application of the composition to the surface. When used, volatile solvents are typically present at levels of from about 2% to about 12% in the compositions.

The hard surface cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 7-12

Liquid Hard Surface Cleaning Compositions

Component	Example No.					
	7	8	9	10	11	12
Gln66Asn	0.05	0.50	0.02	0.03	0.10	0.03
Gly206Asn	–	–	-	-	0.20	0.02
Na_2DIDA*						
EDTA**	–	–	2.90	2.90	–	–
Na Citrate	–	–	–	–	2.90	2.90
NaC_{12} Alkyl-benzene sulfonate	1.95	–	1.95	–	1.95	–
NaC_{12} Alkylsulfate	–	2.20	–	2.20	–	2.20
NaC_{12}(ethoxy)*** sulfate	–	2.20	–	2.20	–	2.20
C_{12} Dimethylamine oxide	–	0.50	–	0.50	–	0.50
Na Cumene sulfonate	1.30	–	1.30	–	1.30	–
Hexyl Carbitol***	6.30	6.30	6.30	6.30	6.30	6.30
Water****	balance to 100%					

*Disodium N-diethyleneglycol-N,N-iminodiacetate

**Na_4 ethylenediamine diacetic acid

***Diethyleneglycol monohexyl ether

****All formulas adjusted to pH 7

In Examples 7-10, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gln66Asn, with substantially similar

results.

In Examples 11-12, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Gln66Asn and Gly206Asn, with substantially similar results.

Examples 13-18
Spray Compositions for Cleaning Hard Surfaces and Removing Household Mildew

Component	Example No.					
	13	14	15	16	17	18
Thr111Asn	0.50	0.05	0.60	0.30	0.20	0.30
Thr217Gly + Ser222Glu	-	-	-	-	0.30	0.10
Sodium octyl sulfate	2.00	2.00	2.00	2.00	2.00	2.00
Sodium dodecyl sulfate	4.00	4.00	4.00	4.00	4.00	4.00
Sodium hydroxide	0.80	0.80	0.80	0.80	0.80	0.80
Silicate (Na)	0.04	0.04	0.04	0.04	0.04	0.04
Perfume	0.35	0.35	0.35	0.35	0.35	0.35
Water	balance to 100%					

Product pH is about 7.

In Examples 13-16, the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr111Asn, with substantially similar results.

In Examples 17-18, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr111Asn and Thr217Gly + Ser222Glu, with substantially similar results.

2.   Dishwashing Compositions

In another embodiment of the present invention, dishwashing compositions comprise one or more enzyme variants of the present invention. As used herein, "dishwashing composition" refers to all forms for compositions for cleaning dishes, including but not limited to, granular and liquid forms. The dishwashing composition embodiment of the present invention is illustrated by the following examples.

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Examples 19-24
Dishwashing Composition

Component	Example No.					
	19	20	21	22	23	24
Gly139Asn	0.05	0.50	0.02	0.40	0.10	0.03
Ser207Glu + Tyr210Ser + Gly223Asn	-	-	-	-	0.40	0.02
C_{12}-C_{14} N-methyl-glucamide	0.90	0.90	0.90	0.90	0.90	0.90
C_{12} ethoxy (1) sulfate	12.00	12.00	12.00	12.00	12.00	12.00
2-methyl undecanoic acid	4.50	4.50	4.50	4.50	4.50	4.50
C_{12} ethoxy (2) carboxylate	4.50	4.50	4.50	4.50	4.50	4.50
C_{12} alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00
C_{12} amine oxide	3.00	3.00	3.00	3.00	3.00	3.00
Sodium cumene sulfonate	2.00	2.00	2.00	2.00	2.00	2.00
Ethanol	4.00	4.00	4.00	4.00	4.00	4.00
Mg^{++} (as $MgCl_2$)	0.20	0.20	0.20	0.20	0.20	0.20
Ca^{++} (as $CaCl_2$)	0.40	0.40	0.40	0.40	0.40	0.40
Water	balance to 100%					

Product pH is adjusted to 7.

In Examples 19-22, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly139Asn, with substantially similar results.

In Examples 23-24, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly139Asn and Ser207Glu + Tyr210Ser + Gly223Asn, with substantially similar results.

3. Fabric cleaning compositions

In another embodiment of the present invention, fabric cleaning compositions comprise one or more enzyme variants of the present invention. As used herein, "fabric cleaning composition" refers to all forms for detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms. Preferred fabric cleaning compositions are those in the liquid form.

a. Granular fabric cleaning compositions

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from

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about 0.005% to about 5%, more preferably from about 0.01% to about 1% by weight of active enzyme of the composition. In addition to one or more enzyme variants, the granular fabric cleaning compositions typically comprise at least one surfactant, one or more builders, and, in some cases, a bleaching agent.

The granular fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 25-28

Granular Fabric Cleaning Composition

Component	Example No.			
	25	26	27	28
Ala168Asn	0.10	0.20	0.03	0.05
Tyr210Gly + Thr212Pro + Pro214Gly + Ser222Asp	-	-	0.02	0.05
C_{13} linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylaenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 25-26, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ala168Asn, with substantially similar results.

In Examples 27-28, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Ala168Asn and Tyr210Gly + Thr212Pro + Pro214Gly + Ser222Asp, with substantially similar results.

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Examples 29-32
Granular Fabric Cleaning Composition

Component	Example No.			
	29	30	31	32
Thr195Pro	0.10	0.20	0.03	0.05
Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu + Thr224Gly	-	-	0.02	0.05
C_{12} alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C_{12}-C_{14} secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 29-30, the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr195Pro, with substantially similar results.

In Examples 31-32, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Thr195Pro and Trp208Phe + Tyr213Met + Thr215Pro + Ser220Glu + Thr224Gly, with substantially similar results.

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Examples 33-36
Granular Fabric Cleaning Composition

	Example No.			
Component	33	34	35	36
Gly67Ser + Gly72Ser	0.10	0.20	0.03	0.05
Tyr171Thr	-	-	0.02	0.05
C_{13} linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylaenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 33-34, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly67Ser + Gly72Ser, with substantially similar results.

In Examples 35-36, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly67Ser + Gly72Ser and Tyr171Thr, with substantially similar results.

Examples 37-40
Granular Fabric Cleaning Composition

	Example No.			
Component	37	38	39	40
Ser192Asp + Phe193Leu + Thr195Asn	0.10	0.20	0.03	0.05
Tyr213Ser + Thr217Gly + Gly223Glu	-	-	0.02	0.05
C_{12} alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C_{12}-C_{14} secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 37-38, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser192Asp + Phe193Leu + Thr195Asn, with substantially similar results.

In Examples 39-40, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser192Asp + Phe193Leu + Thr195Asn and Tyr213Ser + Thr217Gly + Gly223Glu, with substantially similar results.

Examples 41-42
Granular Fabric Cleaning Composition

Component	Example No. 41	Example No. 42
Linear alkyl benzene sulphonate	11.4	10.70
Tallow alkyl sulphate	1.80	2.40
C_{14-15} alkyl sulphate	3.00	3.10
C_{14-15} alcohol 7 times ethoxylated	4.00	4.00
Tallow alcohol 11 times ethoxylated	1.80	1.80
Dispersant	0.07	0.1
Silicone fluid	0.80	0.80
Trisodium citrate	14.00	15.00
Citric acid	3.00	2.50
Zeolite	32.50	32.10
Maleic acid acrylic acid copolymer	5.00	5.00
Diethylene triamine penta methylene phosphonic acid	1.00	0.20
Leu104Asp + Gly139Pro + Tyr171Asp + Ser191Asp + Ser222Glu + Thr224Ser	0.30	0.30
Lipase	0.36	0.40
Amylase	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
Polyvinyl pyrrolidone	0.30	0.50
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.1	0.1
Minors	Up to 100	Up to 100

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Examples 43-44
Granular Fabric Cleaning Composition

Component	Example No. 43	44
Sodium linear C_{12} alkyl benzene-sulfonate	6.5	8.0
Sodium sulfate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
Polyvinyl pyrrolidone	0.5	0.7
Tetraacetylethylene diamine	3.0	3.0
Boric acid	4.0	-
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Pro205Gly + Trp208Leu + Thr217Gln + Leu221Gln + Gly223Gln + Thr224Glu	0.4	0.4
Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 45
Compact Granular Fabric Cleaning Composition

Component	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C_{25} and C_{45} alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Asn66Ser + Ser70Glu + Gly72Asn	0.5
Lipase	0.2
Cellulase	0.2
Tetracetylethylene diamine	6.0

Percarbonate	22.0
Ethylene diamine disuccinic acid	0.3
Suds suppressor	3.5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyril) biphenyl	0.05
Water, Perfume and Minors	Up to 100

Example 46

Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C_{16}-C_{18} alkyl sulfate	1.3
C_{14-15} alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylethylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Ser109Glu + Thr113Gly	0.4
Lipase	0.2
Amylase	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water, perfume and minors	Up to 100

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Example 47
Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C_{14-15} alcohol 7 times ethoxylated	4.4
C_{12-15} alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Gly135Gln + Val138Asp + Gly139Ser	0.2
Lipase	0.36
Cellulase	0.13
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylethylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

b.   Liquid fabric cleaning compositions

-    Liquid fabric cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1%, by weight of active enzyme of the composition. Such liquid fabric cleaning compositions typically additionally comprise an anionic surfactant, a fatty acid, a water-soluble detergency builder and water.

The liquid fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 48-52
Liquid Fabric Cleaning Compositions

Component	Example No.				
	48	49	50	51	52
Asn166Asp + Thr167Asn + Asn170Ser + Tyr171Thr	0.05	0.03	0.30	0.03	0.10
Ser191Glu + Ser192Asp + Phe193Asp + Thr195Pro	-	-	-	0.01	0.20
C_{12}- C_{14} alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C_{10} alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 48-50 the Thermitase variants recited in Tables 2-36, among others, are substituted for Asn166Asp + Thr167Asn + Asn170Ser + Tyr171Thr, with substantially similar results.

In Examples 51-52, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Asn166Asp + Thr167Asn + Asn170Ser + Tyr171Thr and Ser191Glu + Ser192Asp + Phe193Asp + Thr195Pro, with substantially similar results.

Examples 53-57
Liquid Fabric Cleaning Compositions

Component	Example No.				
	53	54	55	56	57
Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Met + Ser220Glu	0.05	0.03	0.30	0.03	0.10
Asn 68Asp + Val138Asp + Ala164Gly + Ala168Glu + Thr217Gly	–	–	–	0.01	0.20
C_{12}- C_{14} alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C_{10} alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 53-55 the Thermitase variants recited in Tables 2-36, among others, are substituted for Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Met + Ser220Glu, with substantially similar results.

In Examples 56-57, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Pro205Gln + Gly206Ser + Trp208Val + Tyr210Leu + Thr212Asn + Tyr218Met + Ser220Glu and Asn 68Asp + Val138Asp + Ala164Gly + Ala168Glu + Thr217Gly, with substantially similar results.

Examples 58-59
Liquid Fabric Cleaning Composition

Component	Example No. 58	59
C_{12-14} alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C_{12-15} alkyl sulphate	8.0	8.0
Sodium sulfate of C_{12-15} alcohol 2 times ethoxylated	-	3.0
C_{12-15} alcohol 7 times ethoxylated	-	8.0
C_{12-15} alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)	0.2	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Leu134Cys + Thr137Glu + Val138Gly	0.2	0.2
Polyvinyl pyrrolidone	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.4	0.1
Waters and minors	up to 100 parts	

In each of Examples 58 and 59 herein, the Thermitase variants recited in Tables 2-36, among others, are substituted for Leu134Cys + Thr137Glu + Val138Gly, with substantially similar results.

Examples 60-62
Liquid Fabric Cleaning Composition

Component	Example No. 60	61	62
Citric Acid	7.10	3.00	3.00
Fatty Acid	2.00	-	2.00
Ethanol	1.93	3.20	3.20
Boric Acid	2.22	3.50	3.50
Monoethanolamine	0.71	1.09	1.09
1,2 Propanediol	7.89	8.00	8.00
NaCumene Sulfonate	1.80	3.00	3.00
NaFormate	0.08	0.08	0.08
NaOH	6.70	3.80	3.80
Silicon anti-foam agent	1.16	1.18	1.18
Gly67Glu + Ser70Asp + Gly72Ser + Thr73Ser	0.0145	-	-
Ile209Ala + Ala219Pro	-	0.0145	-
Leu216Asn	-	-	0.0145
Lipase	0.200	0.200	0.200
Cellulase	-	7.50	7.50
Soil release polymer	0.29	0.15	0.15
Anti-foaming agents	0.06	0.085	0.085
Brightener 36	0.095	-	-
Brightener 3	-	0.05	0.05
C_{12} alkyl benzenesulfonic acid	9.86	-	-
C_{12-15} alkyl polyethoxylate (2.5) sulfate	13.80	18.00	18.00
C_{12} glucose amide	-	5.00	5.00
C_{12-13} alkyl polyethoxylate (9)	2.00	2.00	2.00
Water, perfume and minors	balance to 100%		

c.   <u>Bar fabric cleaning compositions</u>

Bar fabric cleaning compositions of the present invention suitable for hand-washing soiled fabrics contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight of the composition.

The bar fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

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Examples 63-66
Bar Fabric Cleaning Compositions

Component	Example No. 63	64	65	66
Trp112Cys + Thr217Gly	0.3	-	0.1	0.02
Val103Ala + Thr212Ser	-	-	0.4	0.03
C_{12}-C_{16} alkyl sulfate, Na	20.0	20.0	20.0	20.00
C_{12}-C_{14} N-methyl glucamide	5.0	5.0	5.0	5.00
C_{11}-C_{13} alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
$CaSO_4$	1.0	1.0	1.0	1.00
$MgSO_4$	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as $CaCO_3$, talc, clay, silicates, and the like.

In Examples 63-64 the Thermitase variants recited in Tables 2-36, among others, are substituted for Trp112Cys + Thr217Gly, with substantially similar results.

- In Examples 65-66, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Trp112Cys + Thr217Gly and Val103Ala + Thr212Ser, with substantially similar results.

Examples 67-70
Bar Fabric Cleaning Compositions

Component	Example No.			
	67	68	69	70
Ser109Glu	0.3	-	0.1	0.02
Pro169Glu	-	0.3	0.4	0.03
C_{12}-C_{16} alkyl sulfate, Na	20.0	20.0	20.0	20.00
C_{12}-C_{14} N-methyl glucamide	5.0	5.0	5.0	5.00
C_{11}-C_{13} alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
$CaSO_4$	1.0	1.0	1.0	1.00
$MgSO_4$	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as $CaCO_3$, talc, clay, silicates, and the like.

In Example 67, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser109Glu, with substantially similar results.

- In Example 68, the Thermitase variants recited in Tables 2-36, among others, are substituted for Pro169Glu, with substantially similar results.

In Examples 69-70, any combination of the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser109Glu and Pro169Glu, with substantially similar results.

B. <u>Additional Cleaning Compositions</u>

In addition to the hard surface cleaning, dishwashing and fabric cleaning compositions discussed above, one or more enzyme variants of the present invention may be incorporated into a variety of other cleaning compositions where hydrolysis of an insoluble substrate is desired. Such

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additional cleaning compositions include but are not limited to, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning compositions.

1.   Oral cleaning compositions

In another embodiment of the present invention, a pharmaceutically-acceptable amount of one or more enzyme variants of the present invention are included in compositions useful for removing proteinaceous stains from teeth or dentures. As used herein, "oral cleaning compositions" refers to dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Preferably, the oral cleaning compositions comprise from about 0.0001% to about 20% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 10%, more preferably still from about 0.01% to about 5%, by weight of the composition, and a pharmaceutically-acceptable carrier. As used herein, "pharmaceutically-acceptable" means that drugs, medicaments or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

Typically, the pharmaceutically-acceptable oral cleaning carrier components of the oral cleaning components of the oral cleaning compositions will generally comprise from about 50% to about 99.99%, preferably from about 65% to about 99.99%, more preferably from about 65% to about 99%, by weight of the composition.

The pharmaceutically-acceptable carrier components and optional components which may be included in the oral cleaning compositions of the present invention are well known to those skilled in the art. A wide variety of composition types, carrier components and optional components useful in the oral cleaning compositions are disclosed in U.S. Patent 5,096,700, Seibel, issued March 17, 1992; U.S. Patent 5,028,414, Sampathkumar, issued July 2, 1991; and U.S. Patent 5,028,415, Benedict, Bush and Sunberg, issued July 2, 1991; all of which are incorporated herein by reference.

The oral cleaning composition embodiment of the present invention is illustrated by the following examples.

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Examples 71-74
Dentifrice Composition

| Component | Example No. | | | |
	71	72	73	74
Tyr218Ala	2.000	3.500	1.500	2.000
Sorbitol (70% aqueous solution)	35.000	35.000	35.000	35.000
PEG-6*	1.000	1.000	1.000	1.000
Silica dental abrasive**	20.000	20.000	20.000	20.000
Sodium fluoride	0.243	0.243	0.243	0.243
Titanium dioxide	0.500	0.500	0.500	0.500
Sodium saccharin	0.286	0.286	0.286	0.286
Sodium alkyl sulfate (27.9% aqueous solution)	4.000	4.000	4.000	4.000
Flavor	1.040	1.040	1.040	1.040
Carboxyvinyl Polymer***	0.300	0.300	0.300	0.300
Carrageenan****	0.800	0.800	0.800	0.800
Water	balance to 100%			

*PEG-6 = Polyethylene glycol having a molecular weight of 600.

**Precipitated silica identified as Zeodent 119 offered by J.M. Huber.

***Carbopol offered by B.F. Goodrich Chemical Company.

****Iota Carrageenan offered by Hercules Chemical Company.

In Examples 71-74 the Thermitase variants recited in Tables 2-36, among others, are substituted for Tyr218Ala, with substantially similar results.

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Examples 75-78
Mouthwash Composition

Component	Example No.			
	75	76	77	78
Ala164His + Ser220Glu	3.00	7.50	1.00	5.00
SDA 40 Alcohol	8.00	8.00	8.00	8.00
Flavor	0.08	0.08	0.08	0.08
Emulsifier	0.08	0.08	0.08	0.08
Sodium Fluoride	0.05	0.05	0.05	0.05
Glycerin	10.00	10.00	10.00	10.00
Sweetener	0.02	0.02	0.02	0.02
Benzoic acid	0.05	0.05	0.05	0.05
Sodium hydroxide	0.20	0.20	0.20	0.20
Dye	0.04	0.04	0.04	0.04
Water	balance to 100%			

In Examples 75-78, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ala164His + Ser220Glu, with substantially similar results.

Examples 79-82
Lozenge Composition

Component	Example No.			
	79	80	81	82
Leu221Val + Thr224Gln	0.01	0.03	0.10	0.02
Sorbitol	17.50	17.50	17.50	17.50
Mannitol	17.50	17.50	17.50	17.50
Starch	13.60	13.60	13.60	13.60
Sweetener	1.20	1.20	1.20	1.20
Flavor	11.70	11.70	11.70	11.70
Color	0.10	0.10	0.10	0.10
Corn Syrup	balance to 100%			

In Examples 79-82, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser Leu221Val + Thr224Gln, with substantially similar results.

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Examples 83-86
Chewing Gum Composition

Component	Example No.			
	83	84	85	86
Ser191Asp + Phe193Ile + Thr195Gly	0.03	0.02	0.10	0.05
Sorbitol crystals	38.44	38.40	38.40	38.40
Paloja-T gum base*	20.00	20.00	20.00	20.00
Sorbitol (70% aqueous solution)	22.00	22.00	22.00	22.00
Mannitol	10.00	10.00	10.00	10.00
Glycerine	7.56	7.56	7.56	7.56
Flavor	1.00	1.00	1.00	1.00

*Supplied by L.A. Dreyfus Company.

In Examples 83-86, the Thermitase variants recited in Tables 2-36, among others, are substituted for Ser191Asp + Phe193Ile + Thr195Gly, with substantially similar results.

2.   Denture cleaning compositions

In another embodiment of the present invention, denture cleaning compositions for cleaning dentures outside of the oral cavity comprise one or more enzyme variants of the present invention. Such denture cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.0001% to about 50% of one or more of the enzyme variants, more preferably from about 0.001% to about 35%, more preferably still from about 0.01% to about 20%, by weight of the composition, and a denture cleansing carrier. Various denture cleansing composition formats such as effervescent tablets and the like are well known in the art (see for example U.S. Patent 5,055,305, Young, incorporated herein by reference), and are generally appropriate for incorporation of one or more of the enzyme variants for removing proteinaceous stains from dentures.

The denture cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 87-90
Two-layer Effervescent Denture Cleansing Tablet

| | Example No. | | | |
Component	87	88	89	90
Acidic Layer				
Gly135Gln + Gly139Asn + Asn140Gln + Ser141Asp	1.0	1.5	0.01	0.05
Tartaric acid	24.0	24.0	24.00	24.00
Sodium carbonate	4.0	4.0	4.00	4.00
Sulphamic acid	10.0	10.0	10.00	10.00
PEG 20,000	4.0	4.0	4.00	4.00
Sodium bicarbonate	24.5	24.5	24.50	24.50
Potassium persulfate	15.0	15.0	15.00	15.00
Sodium acid pyrophosphate	7.0	7.0	7.00	7.00
Pyrogenic silica	2.0	2.0	2.00	2.00
TAED*	7.0	7.0	7.00	7.00
Ricinoleylsulfosuccinate	0.5	0.5	0.50	0.50
Flavor	1.0	1.0	1.00	1.00
Alkaline Layer				
Sodium perborate monohydrate	32.0	32.0	32.00	32.00
Sodium bicarbonate	19.0	19.0	19.00	19.00
EDTA	3.0	3.0	3.00	3.00
Sodium tripolyphosphate	12.0	12.0	12.00	12.00
PEG 20,000	2.0	2.0	2.00	2.00
Potassium persulfate	26.0	26.0	26.00	26.00
Sodium carbonate	2.0	2.0	2.00	2.00
Pyrogenic silica	2.0	2.0	2.00	2.00
Dye/flavor	2.0	2.0	2.00	2.00

*Tetraacetylethylene diamine

In Examples 87-90, the Thermitase variants recited in Tables 2-36, among others, are substituted for Gly135Gln + Gly139Asn + Asn140Gln + Ser141Asp, with substantially similar results.

3.   Contact Lens Cleaning Compositions

In another embodiment of the present invention, contact lens cleaning compositions comprise one or more enzyme variants of the present invention. Such contact lens cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.01%

to about 50% of one or more of the enzyme variants, more preferably from about 0.01% to about 20%, more preferably still from about 1% to about 5%, by weight of the composition, and a contact lens cleaning carrier. Various contact lens cleaning composition formats such as tablets, liquids and the like are well known in the art (see for example U.S. Patent 4,863,627, Davies, Meaken and Rees, issued September 5, 1989; U.S. Patent Re. 32,672, Huth, Lam and Kirai, reissued May 24, 1988; U.S. Patent 4,609,493, Schäfer, issued September 2, 1986; U.S. Patent, 4,690,793, Ogunbiyi and Smith, issued September 1, 1987; U.S. Patent 4,614,549, Ogunbiyi, Riedhammer and Smith, issued September 30, 1986; and U.S. Patent 4,285,738, Ogata, issued August 25, 1981; each of which are incorporated herein by reference), and are generally appropriate for incorporation of one or more enzyme variants of the present invention for removing proteinaceous stains from contact lens.

The contact lens cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 91-94
Enzymatic Contact Lens Cleaning Solution

Component	Example No.			
	91	92	93	94
Leu221Gln	0.01	0.5	0.1	2.0
Glucose	50.00	50.0	50.0	50.0
Nonionic surfactant (polyoxyethlene-polyoxypropylene copolymer)	2.00	2.0	2.0	2.0
Anionic surfactant (polyoxyethylene-_ alkylphenylether sodium sulfricester)	1.00	1.0	1.0	1.0
Sodium chloride	1.00	1.0	1.0	1.0
Borax	0.30	0.3	0.3	0.3
Water	balance to 100%			

In Examples 91-94, the Thermitase variants recited in Tables 2-36, among others, are substituted for Leu221Gln, with substantially similar results.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing

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from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: BRODE, PHILIP F.
                 BARNETT, BOBBY L.
                 RUBINGH, DONN N.

    (ii) TITLE OF INVENTION: THERMITASE VARIANTS HAVING DECREASED
           ADSORPTION AND INCREASED HYDROLYSIS

    (iii) NUMBER OF SEQUENCES: 1

    (iv) CORRESPONDENCE ADDRESS:
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    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: CORSTANJE, BRAHM J.
        (B) REGISTRATION NUMBER: 34,804
        (C) REFERENCE/DOCKET NUMBER: 5607

    (ix) TELECOMMUNICATION INFORMATION:
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        (B) TELEFAX: (513) 627-0260

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 279 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Thr Pro Asn Asp Pro Tyr Phe Ser Ser Arg Gln Tyr Gly Pro
Gln
1   5   10   15

Lys Ile Gln Ala Pro Gln Ala Trp Asp Ile Ala Glu Gly Ser Gly
Ala
            20   25   30

Lys Ile Ala Ile Val Asp Thr Gly Val Gln Ser Asn His Pro Asp
Leu
            35   40   45

Ala Gly Lys Val Val Gly Gly Trp Asp Phe Val Asp Asn Asp Ser
Thr

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                50                      55                      60
        Pro Gln Asn Gly Asn Gly His Gly Thr His Cys Ala Gly Ile Ala
Ala
        65                      70                      75              80
        Ala Val Thr Asn Asn Ser Thr Gly Ile Ala Gly Thr Ala Pro Lys
Ala
                        85                      90                      95
        Ser Ile Leu Ala Val Arg Val Leu Asp Asn Ser Gly Ser Gly Thr
Trp
                    100                     105                     110
        Thr Ala Val Ala Asn Gly Ile Thr Tyr Ala Ala Asp Gln Gly Ala
Lys
                    115                     120                     125
        Val Ile Ser Leu Ser Leu Gly Gly Thr Val Gly Asn Ser Gly Leu
Gln
                    130                     135                     140
        Gln Ala Val Asn Tyr Ala Trp Asn Lys Gly Ser Val Val Val Ala
Ala
        145                     150                     155
160
        Ala Gly Asn Ala Gly Asn Thr Ala Pro Asn Tyr Pro Ala Tyr Tyr
Ser
                    165                     170                     175
        Asn Ala Ile Ala Val Ala Ser Thr Asp Gln Asn Asp Asn Lys Ser
Ser
                    180                     185                     190
        Phe Ser Thr Tyr Gly Ser Val Val Asp Val Ala Ala Pro Gly Ser
Trp
                    195                     200                     205
        Ile Tyr Ser Thr Tyr Pro Thr Ser Thr Tyr Ala Ser Leu Ser Gly
Thr
                    210                     215                     220
        Ser Met Ala Thr Pro His Val Ala Gly Val Ala Gly Leu Leu Ala
Ser
        225                     230                     235
240
        Gln Gly Arg Ser Ala Ser Asn Ile Arg Ala Ala Ile Glu Asn Thr
Ala
                    245                     250                     255
        Asp Lys Ile Ser Gly Thr Gly Thr Tyr Trp Ala Lys Gly Arg Val
Asn
                    260                     265                     270
        Ala Tyr Lys Ala Val Gln Tyr
                    275
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What is Claimed is:

1.   A Thermitase variant having a modified amino acid sequence of Thermitase wild-type amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises a substitution at one or more positions in one or more of the loop regions; wherein

    A.   when a substitution occurs in the first loop region, the substitution occurs at one or more of positions 66, 69, 70, 72 or 73; wherein

        a.   when a substitution occurs at position 66, the substituting amino acid is Asn;

        b.   when a substitution occurs at position 69, the substituting amino acid is Gln;

        c.   when a substitution occurs at position 70, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

        d.   when a substitution occurs at position 72, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and

        e.   when a substitution occurs at position 73, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    B.   when a substitution occurs in the second loop region, the substitution occurs at one or more of positions 103, 104, 105, 106, 108, 110, 112, 113 or 114; wherein

        a.   when a substitution occurs at position 103, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

        b.   when a substitution occurs at position 104, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Gly, His, Ile, Met, Pro or Ser;

        c.   when a substitution occurs at position 105, the substituting amino acid is Glu;

        d.   when a substitution occurs at position 106, the substituting amino acid is Gln;

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    e.   when a substitution occurs at position 108, the substituting amino acid is Asn, Gln, Pro or Ser;

    f.   when a substitution occurs at position 110, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    g.   when a substitution occurs at position 112, the substituting amino acid is Asn, Asp, Cys, Gln, Glu, His, Ile, Met, Phe, Pro, Thr or Tyr;

    h.   when a substitution occurs at position 113, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and

    i.   when a substitution occurs at position 114, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

C.   when a substitution occurs in the third loop region, the substitution occurs at one or more of positions 134, 135, 136, 137, 138, 139 or 141; wherein

    a.   when a substitution occurs at position 134, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Met, Pro, Ser, Thr or Val;

    b.   when a substitution occurs at position 135, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    c.   when a substitution occurs at position 136, the substituting amino acid is Pro;

    d.   when a substitution occurs at position 137, the substituting amino acid is Asn, Asp, Gln, Glu or Ser;

    e.   when a substitution occurs at position 138, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

    f.   when a substitution occurs at position 139, the substituting amino acid is Asn, Gln, Pro or Ser; and

    g.   when a substitution occurs at position 141, the substituting amino acid is Asp or Glu;

D.   when a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 167, 169, or 171; wherein

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    a.   when a substitution occurs at position 167, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    b.   when a substitution occurs at position 169, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser; and

    c.   when a substitution occurs at position 171, the substituting amino acid is His, Ile, Leu, Met or Pro;

E.   when a substitution occurs in the fifth loop region, the substitution occurs at one or more of position 193; wherein

    a.   when a substitution occurs at position 193, the substituting amino acid is Asn, Cys, Gln, His, Ile, Met, Thr or Tyr;

F.   when a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 220, 221, 223 or 224; wherein

    a.   when a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

    b.   when a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;

    c.   when a substitution occurs at position 206, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    d.   when a substitution occurs at position 207, the substituting amino acid is Asp or Glu;

    e.   when a substitution occurs at position 208, the substituting amino acid is Asp or Glu;

    f.   when a substitution occurs at position 209, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;

    g.   when a substitution occurs at position 210, the substituting amino acid is Asp, His, Ile, Leu, Met or Pro;

    h.   when a substitution occurs at position 211, the substituting amino acid is Asp or Glu;

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i.   when a substitution occurs at position 212, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

j.   when a substitution occurs at position 213, the substituting amino acid is Asp, Glu, His, Ile, Leu, Met, Phe, Pro or Val;

k.   when a substitution occurs at position 214, the substituting amino acid is Asn, Gln, Gly, or Ser;

l.   when a substitution occurs at position 215, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

m.   when a substitution occurs at position 216, the substituting amino acid is Asp or Glu;

n.   when a substitution occurs at position 217, the substituting amino acid is Asp, Glu or Pro;

o.   when a substitution occurs at position 220, the substituting amino acid is Asp or Glu;

p.   when a substitution occurs at position 221, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;

q.   when a substitution occurs at position 222, the substituting amino acid is Asp or Glu;

r.   when a substitution occurs at position 223, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and

s.   when a substitution occurs at position 224, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

whereby the Thermitase variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type Thermitase.

2.   The Thermitase variant of Claim 1, wherein one or more substitutions occur in the first loop region.

3.   The Thermitase variant of Claim 1, wherein one or more substitutions occur in the second loop region.

4.   The Thermitase variant of Claim 1, wherein one or more substitutions occur in the third loop region.

5.   The Thermitase variant of Claim 1, wherein one or more substitutions occur in the fourth loop region.

6.   The Thermitase variant of Claim 1, wherein one or more substitutions occur in the fifth loop region.

7.   The Thermitase variant of Claim 1, wherein one or more substitutions occur in the sixth loop region.

8.   A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, characterized in that the cleaning composition comprises the Thermitase variant of any of Claims 1-7 and a cleaning composition carrier; preferably the cleaning composition is a hard surface cleaning composition or the cleaning composition is a fabric cleaning composition; preferably the composition comprises at least about 5% surfactant and at least about 5% builder, by weight of the composition; preferably the composition further comprises cleaning composition materials selected from the group consisting of solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds suppressors, fabric softeners, suds boosters, enzyme stabilizers, bleaching agents, dyes, perfumes, and mixtures thereof.

9.   A DNA sequence encoding the Thermitase variant of any of Claims 1-7.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/57   C12N9/52   C11D7/42   C12N1/21   A61K7/28
         //(C12N1/21,C12R1:125)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N   C11D   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 00345 (NOVONORDISK AS) 10 January 1991 see the whole document ---	1-9
Y	EP,A,0 405 901 (UNILEVER PLC ;UNILEVER NV (NL)) 2 January 1991 see the whole document ---	1-9
Y	EP,A,0 405 902 (UNILEVER PLC ;UNILEVER NV (NL)) 2 January 1991 see the whole document ---	1-9
Y	EP,A,0 380 362 (GENEX CORP) 1 August 1990 see the whole document ---	1-9

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[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 July 1996	25.07.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hix, R

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	ACTA BIOLOGICA ET MEDICA GERMANICA, vol. 41, no. 2/3, 1982, pages 137-144, XP000574173 G. HANSEN ET AL.: "Thermitase, eine thermostabile Serin-Protease aus Thermoactinomyces vulgaris: Wechselwirkung zwischen acktivem Zentrum und SH-Gruppe des Enzyms" see the whole document ---	
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